Gerhard Talsky

Derivative Spectrophotometry

Low and Higher Order

Derivative Spectrophotometry. By Gerhard Talsky

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Preface

This book is intended to provide an introduction to low- and higher-order derivative spectrophotometry for chemists, biochemists, food chemists, pharmacists, and physicists, as well as industrial chemists and other scientists dealing with the quantitative estimation and analysis of substances. To this date the topic has been reviewed in the literature, but as of yet no specific handbook or monograph exists that provides the theoretical background and the practical instructions necessary for using this highly efficient analytical technique.

Although various mechanical, optical, and electrical methods for differentiation of electrical signals have already been in use for about 35 years, derivative spectrophotometry had no common application until recently. Generally, the first- and second-order derivative spectra were employed; fourth-order derivatives, generated with digital computers, were only used in a few special cases. Thanks to the progress made in modern electronics, most commercial spectrophotometers available for the last ten years have been fitted with derivative modules. A closer study of these derivative spectra has shown that many previously intractable analytical problems can only be solved by higher-order differentiation ($n > 2$) of signals.

In this text a theoretical and practical introduction is given for anyone interested in applying derivative spectrophotometry – or other multidifferentiation techniques – to analytical studies. In particular, the various modes of instrumentation for signal differentiation are described and critically compared to point out the advantages and drawbacks of the various methods and to reveal common difficulties.

The book is divided into five chapters. In Chapter 1, an introduction to this analytical method is provided; in Chapter 2, theoretical considerations are covered; in Chapter 3, the instrumentation for the generation of low- and higher-order derivatives is described; in Chapter 4, practical aspects of the technique are considered; and in Chapter 5 numerous applications are cited to stimulate readers to use this technique in their own experiments. Instead of an exhaustive discussion of the more than 1200 published papers containing preferential treatment of *low*-order derivatives, the advantages of *higher*-order derivatives are presented, with carefully selected examples cited in the literature. Further examples have been created in our laboratory with the help of analog and digital computers over the last sixteen years. I hope that this monograph will serve to further expand the use of *higher-order* derivative spectrophotometry.

Special thanks go to the collaborators who took part in the basic investigations and practical applications of higher-order derivatives: research chemists Lothar May-

ring, Hans Kreuzer, Sybill Götz-Maler, Margot Glasbrenner, Jürgen Gottschlich, Otto Haubensak, Sibylle Tichy, Georgios Gianitsopoulos, Maja Ristić-Šolajić, Robert Schmid, Xiashi Zhu, and the technical assistant Franziska Reisinger. Finally, I acknowledge the invaluable help of my wife Sophie Maria for typing and proofreading the manuscript.

Munich, Autumn 1994 G. Talsky

Contents

List of Symbols and Abbreviations

A	absorbance
A_0	amplitude of the main peak
A^{I}, A^{II}...	first, second derivative
$A_{\max}$	max. absorbance
$A_{(\mathrm{Ref})}$	reflective absorbance
A_λ, $\mathrm{A}_{\mathrm{D}\lambda}$	amplitude of the signal and disturbing signal at wavelength λ
A_{λ_n}	absorbance at the wavelength λ_n
$a_{(\mathrm{AX})}$	auto-correlation coefficient
a_0	coefficient
a_n, b_n	coefficients of the Fourier transformation
B	apparatus spectral band width
b	band width
C	capacitor
C	capacitance
C_{FWHM}	constant in equation for computing x_σ
C_σ	constant in equation for computing x_{FWHM}
c	concentration or molar concentration
c_0	velocity of light in vacuum
D^n	$D^n = d^n + U^n$ nth derivative of peak (d^n) and background polynome U^n
d	thickness
d	vibration range
d	differentation width
d_{c}	critical distance of overlapping
d^n	nth derivative order
E	energy
E_{ee}	excitation energy of electrons
E_n	Energy of the nth state
E_{rot}, E_{vib}	rotational and vibrational transition energy
ΔE_{el}	exciting energy of electrons
f	function (general)
f	frequency
f	differentiation ratio
f	focal length

f_c	critical frequency
f_{ch}, f_{cl}	upper and low cut-off frequency
f_e	relation of: area exit port/inner area sphere
f_h	highest frequency
f_t	relation: area of all ports/area sphere
H	peak height
h	Planck constant
I, I_λ	intensity of light
$I_{0,\lambda}$	initial intensity of light
$i\ (V)$	i voltage data
L	inductor
L	self-inductance
l	pathlength
N	width of smoothing
N	noise
n	refractive index
P	pole
P_n, P_n	positive extrema and distance of positive extrema
P	spectral reflectance of sphere coating
r	smoothing ratio, that is the ratio of smoothing width to FWHM
R	resistor
R	resistance
R	$R = A_2/A_1$ ratio of amplitudes
R	reflectivity or reflection factor
R_0	diffuse reflectivity
R_∞	reflectivity of a sample with $d = \infty$
R'_∞	relative directional reflectance
R_d, R_i, R_r	diffuse, integral and regular reflectance
R_g	reflectivity of the ground with $d = 0$
R_D	resolution of the differentiator
R_n	ratio of the satellite height to the main peak
S	sign (plus or minus)
s	seconds
s	rate of the wavelength scan
S	main signal height
S	scan velocity
S, S^I, $S^{II}\ldots$	sum of two derivatives
T	transmittance
T_λ	transmittance for the wavelength λ
t	time
t	distance of signal peaks in the fundamental curve
t	throughput (ratio of light flux exiting a sphere to that entering the sphere)
t_1, t_2, $t_3 \ldots t_s$	peak distance of the superposed peaks
u	complex potential (DC and AC)
U	voltage

U_i, U_o	input and output voltage
U_0, U^I, U^{II}, U^n	derivatives of polynomials of the background
V	amplification
V	voltage (unit)
V_a	ratio of amplification
x	$x = \lambda - \lambda_{max}$; distance of λ from peak maximum λ_{max}
Δx	distance of data points
x_{FWHM}	full width at half maximum amplitude
x_σ	halfwidth between the points of inflection
$\bar{y}_n$, $\bar{y}_i$	smoothed ordinate data
z	$\lambda - \lambda_{max}$
α_i, α_r	angle of incident and reflected beam
Δ	shift, or difference
δ	angle of refraction
γ	glossy angle
ε, ε_λ	molar absorption coefficient, general and at wavelength λ
ε'	molar extinction coefficient
λ	wavelength
λ_0	main wavelength
λ_{max}	wavelength of peak maximum
$\Delta\lambda$	λ-shift
ν	frequency
$\tilde{\nu}$	wavenumber
σ	band halfwidth at the point of inflection
τ	time constant
ω	angular velocity
φ	ratio of two FWHMs
A	analog
AC	alternating current
AAS	atomic absorption spectroscopy
AD	analog digital
ADT	analog differentiative type
AM	amplitude modulation
AR	area
CH	chymotrypsin
CHG	chymotrypsinogen
CMC	carboxy methylcellulose
corr.	corrected
D	digital
DC	direct current
DA	digital-analog
DMSO	dimethyl sulfoxide
D^0	Zeroth derivative (= fundamental signal)
EDP	electronic data processing
EPPR	extended peak-peak ratio
ESR	Electron spin resonance

FM	frequency modulated
FT	Fourier transformation
FWHM	full width at half maximum
$FWHM_n$	full width at half maximum for nth differentiation
GC	gas chromatography
GLC	gas liquid chromatography
H	hybrid
HODS	higher-order derivative spectrophotometry ($n > 2$)
HWG	half wave graphical method
HPLC	high-pressure liquid chromatography
IC	integrated circuit
IP	isosbestic point
IP	inflection point
IR	infrared
LC	liquid chromatography
LTH	layer thickness
MD	mode of differentiation
M method	multiplicative method
MO	molecular orbital
NA	numeric aperture
NIR	near infrared region
NMR	nuclear magnetic resonance
OP	operational amplifier
PC	personal computer
PC	puls code
PCM	pulse code modulator
PG	pigment green
PP	point-point differentiation
PP	peak-peak method
PPR	peak-peak ratio method
PT	peak-tangent method
PZ	peak-zero method
RAM	random-access memory
RNase	ribonuclease
SG	Savitzky-Golay method
SNR	signal-to-noise ratio
SNR_n	signal-to-noise ratio for nth differentiation
SPS	side-peak-side method
SPSR	side-peak-side ratio
SSR	signal-to-signal ratio
TLB 6000	analog differentiative type
TLC	thin layer chromatography
UV	ultraviolet
UV-VIS	Ultraviolet-Visible
VIS	visible
VUV	vacuum ultraviolet

1 Introduction

1.1 Development of the Derivative Technique

The method of resolving the fine structure of electric signals by differentiation is about 70 years old. In the early 1920s Lord Rutherford suggested the first derivative technique for the detection of discontinuities in mass spectrometric studies of gas-excitation potentials [1].

This method sank into oblivion until 1953, when Singleton and Collier [2, 3] took advantage of developments in electronics and built the earliest derivative spectrophotometer. They modified an IR spectrophotometer by an analog device to generate second-order spectra and took out a patent for this concept [2]. At the same time, Hammond and Price [4] proposed the wavelength modulation principle (cf. Section 3.3.1), and Giese and French [5] adapted it to study first-order derivative visible spectra of photosynthetic systems.

Also in 1953, Morrison [6] computed first and second-order derivatives (d^1 and d^2) by differential curves $\Delta i(V)/\Delta V$. They were obtained by subtraction in small intervals ($\Delta V = 0.05\ V$) and are close approximations of the true derivatives. However, the complexity of the instrumentation and the unsatisfactory signal-to-noise ratio (SNR) of the electronic devices led all but a few scientists to ignore this method [7]. In 1966 Meister [8] developed a practical resistance-capacity circuit (RC circuit) for the second derivative (d^2), with which he, as well as other photochemists [9, 10], successfully investigated problems in plant photochemistry.

In 1953 Singleton and Collier [2] had already suggested that derivatives higher than second order should give narrower bandwidths; however, they were unable to submit experimental proof because at that time their equipment was not sufficiently sophisticated for the task. Martin [11] confirmend the theoretical basis for computed Lorentzian bands and in 1968 Morrey [12] generated first- to fourth-order derivatives of Gaussian, Student T_3, and Lorentzian distribution functions with a digital computer. Morrey was also able to show that fourth-order derivatives give greater resolution of overlapping bands of synthetic mixtures than second-order derivatives. Savitzky and Golay used least-squares procedures for the smoothing and differentiation of fluctuating data [13]. Butler and Hopkins [14, 15] extended the computer-based approach to the routine generation of second- and fourth-order derivative spectra in order to study photosynthetic materials.

Derivative Spectrophotometry. By Gerhard Talsky

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Higher-order derivative spectra ($n > 2$; HODS) have been systematically and intensively explored in our laboratory [16–25]. In 1978 we were the first to produce high-qualitiy UV-VIS-derivative spectra up to the ninth order, measured on-line and immediately generated by means of a newly developed, low-noise derivative unit with RC circuits, low-pass filters, and smoothing modules. Fell [26] used two second-order analog derivative devices, connected on-line, in order to produce derivatives. Butler [15] computed the sixth- and eighth-order spectra by digital methods, and Sasaki [27] used digital methods to study the superposed derivative spectra of dyes up to the 13th order.

Over the years the quality of commercially available derivative devices has improved. At present, practically all of the new spectrophotometers are commercially fitted with at least second-order derivative systems, but most can go up to the fourth order, and some even up to the sixth or ninth order. This makes it possible to apply the HODS technique not only in research laboratories, but also in routine operations.

1.2 Electron Excitation Spectra

Although differentiation is suited for fine resolution of electric signals in general, this book mainly employs the derivative method for *UV-VIS spectra.* These spectra frequently give broad, poorly defined peaks and, therefore, need to be deconvoluted by differentiation. Molecules that interact with the electric vector of an electromagnetic wave in the visible and ultraviolet region of the electromagnetic spectrum are observed by optical spectroscopy using devices such as prisms, lenses, gratings, or mirrors. The theory behind optical spectroscopy is explained by principles of quantum mechanics.

Absorption of energy takes place when the frequency ν of the incident light is exactly equal to the energy difference of two energy states, e.g., E_1 and E_0, where E_0 is the ground state and E_1 an excited state.

$$h\nu = E_1 - E_0 = h\frac{c_0}{\lambda} \tag{1-1}$$

h: Planck constant
c_0: velocity of light in a vacuum
λ: wavelength of the electromagnetic radiation

Electron excitation and excitation of molecular vibrations and rotations are among the main types of energy transitions. The energy of most molecular vibrations lies in the Infrared region of the electromagnetic spectrum. The signals between 2.5 and 15 μm (2500 to 15000 nm) are especially suited for analytical applications. Higher energies are necessary though, for electron excitation. Accordingly, the wavelength λ of incident light must be short, and the frequency ν and the wave number $\tilde{\nu}$, high, as shown by Eq. (1-1) and the following equations:

$$\lambda = \frac{c_0}{\nu} \tag{1-2}$$

and

$$\tilde{\nu} = \frac{1}{\lambda} = \frac{\nu}{c_0} \tag{1-3}$$

A brief overview of molecular orbital theory will shed light on the nature of electron transitions. The following five molecular orbitals (MOs) are of chief significance:

σ (bonding)	σ^* (antibonding)
π (bonding)	π^* (antibonding)
n (nonbonding).	

Single bonds are described by σ orbitals. Electrons in σ orbitals are much more strongly bound than those in π orbitals of double bonds. Therefore, σ electrons are more difficult to excite than π electrons, by bicentric or polycentric molecular orbitals. Some molecules have atoms with n orbitals (eg, O, N, Br, I). Their electrons do not participate in any bonding. In Fig. 1-1, the energy levels of the orbitals and the various electron transitions are presented schematically.

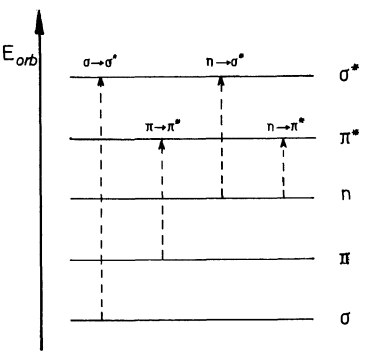

Figure 1-1. Electron orbital transitions.

σ electrons (e.g., in the Single bonds of C–C and C–H) absorb light in the far UV range with $\lambda < 180$ nm, (vacuum UV, VUV), while n and π transitions take place if $\lambda > 180$ nm (Table 1-1).

Ions of transition metals which have incomplete d subshells are able to form complexes with many ions or polar molecules. They are often intensively colored. The middle, or weak, absorption bands in the visible range of the spectrum ($\varepsilon < 10^2$ L mol^{-1}cm^{-1}) are due to the transitions between the orbitals of the central ion. The more intense bands with shorter wavelengths are caused by electron transitions between the central ion and the ligands *(charge-transfer transitions)* or by $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions in the ligands of the transition metal complexes *(inner-ligand transitions)*. In inorganic anions, electron transitions are also possible: Their absorption bands lie in the UV-VIS region.

Table 1-1. Electron transitions of some chromophores, λ_{max} and region of the spectra.

Electron transition	Chromophore	λ_{max} [nm][a]	Region of spectrum[b]
$\sigma \rightarrow \sigma^*$	$-\overset{\vert}{\underset{\vert}{C}}-H$	≈ 120–135	VUV
	$-\overset{\vert}{\underset{\vert}{C}}-\overset{\vert}{\underset{\vert}{C}}-$	≈ 130–150	VUV
$\pi \rightarrow \pi^*$	$-\overset{\vert}{\underset{\vert}{C}}=\overset{\vert}{\underset{\vert}{C}}-$	≈ 160–200	VUV
$n \rightarrow \sigma^*$	$-\overset{\vert}{\underset{\vert}{C}}-\overline{\underline{O}}-$	≈ 170–190	VUV
	$-\overline{\underline{N}}-$	≈ 190–200	UV
	$-\overline{\underline{S}}-$	≈ 190–200 ≈ 220–240	UV UV
	$-\overline{\underline{S}}-\overline{\underline{S}}-$	≈ 190–200 ≈ 240–260	UV UV
	$-\overset{\vert}{\underset{\vert}{C}}-\overline{\underline{Cl}}\vert$	≈ 170–190	VUV
	$-\overset{\vert}{\underset{\vert}{C}}-\overline{\underline{Br}}\vert$	≈ 200–220	UV
	$-\overset{\vert}{\underset{\vert}{C}}-\overline{\underline{I}}\vert$	≈ 250–270	UV
$n \rightarrow \pi^*$	$\overset{\vert}{\underset{\vert}{C}}=\overline{\underline{O}}$	≈ 265–285 vw	UV
	$-\overset{\vert}{\underset{\vert\,O\,\vert}{C}}=\overline{\underline{O}}$	≈ 195–225	UV
$\pi \rightarrow \pi^*$ (conjugated)	$R_1(-\overset{\vert}{\underset{\vert}{C}}=\overset{\vert}{\underset{\vert}{C}})_n-R_2$		
	$n = 1$	≈ 160 s	VUV
	$n = 2$	≈ 220 s	UV
	$n = 3$	≈ 260 s	UV
	$n = 4$	≈ 290 s	UV
	$n = 5$	≈ 320 s	UV
	$n = 6$	≈ 345 s	VIS
	Benzene	≈ 230–270 w ≈ 195–205 m ≈ 180–185 s	UV UV VUV

[a] s: strong ($\varepsilon > 10000$); m: middle (ε 1000–10000); w: weak (ε 100–1000); vw: very weak ($\varepsilon < 100$).

[b] VUV: vacuum ultraviolet; UV: ultraviolet; VIS: visible.

The pure excitation of electrons ($\Delta E_{el} > 1.5$ eV) should give sharp lines (Fig. 1-2a); however, electron excitation is always associated with molecular vibration and rotation (Fig. 1-2b and Fig. 1-2c):

$$E = E_{ee} + E_{vib} + E_{rot} \tag{1-4}$$

$$|E_{ee}| \gg |E_{vib}| \gg |E_{rot}| \tag{1-5}$$

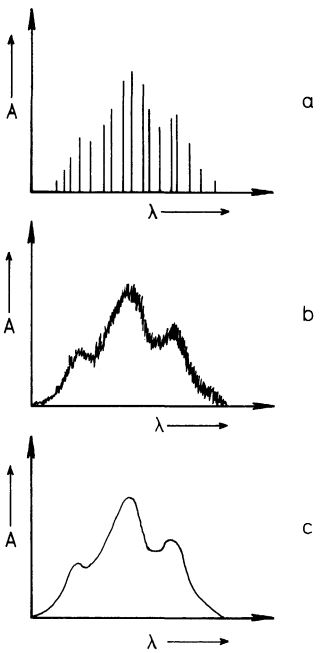

Figure 1-2. UV-Vis spectra.
a) Sharp lines of electron excitation; b) Overlapping of electron signals due to molecular vibration and rotation (gaseous conditions); c) Overlapping signals in solution (schematic drawing).

Therefore, in contrast to the generally sharper signals of IR spectra (Fig. 1-3), the UV-VIS spectra of most liquids, solutions, and solids show broad bands with indistinct shoulders (Fig. 1-4), and their fine structure is suppressed. This is due to intermolecular interactions (including interactions with the solvent molecules), which hinder molecular rotation and vibration. In the gas phase the different absorption peaks (Fig. 1-5) can sometimes be resolved.

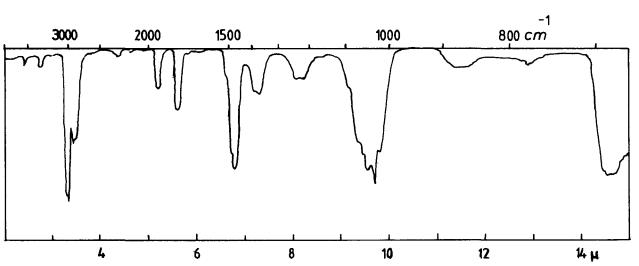

Figure 1-3. IR spectrum of benzene (thin film between KBr crystals).

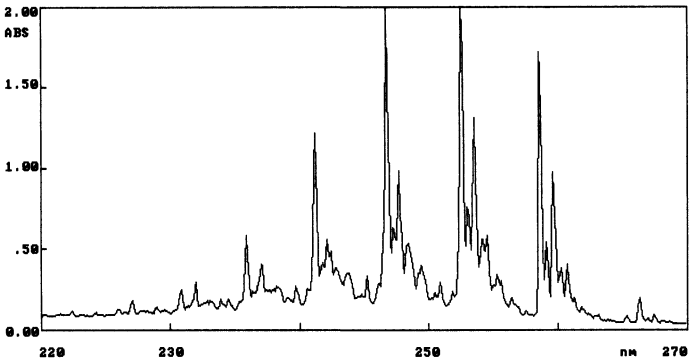

Figure 1-4. UV spectrum of benzene (gas phase; slit width: 0.1 nm).

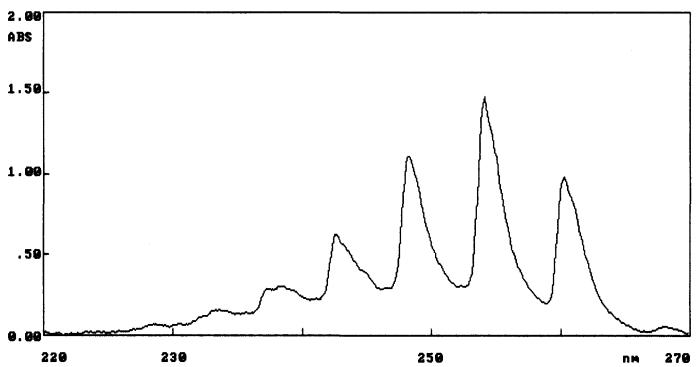

Figure 1-5. UV spectrum of benzene in cyclohexane (slit width: 0.1 nm).

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2 Theoretical Considerations

2.1 Fine Resolution of Spectra

There are several methods for fine resolution of convoluted spectra. The most important techniques are treated briefly in this section.

2.1.1 Optical Methods

The slit of the monochromator limits the band width of the light source. Therefore, minimizing the slit increases the spectral resolution linearly, but the light energy decreases with the second power. Lengthening the optical bank also increases resolution linearly. In turn, the energy decreases with the second power of the distance between the source of light and the multiplier. Of course, the more lines there are per mm of grating, the higher the resolution will be; the practical limit is reached, however, at about 2000–2400 lines per mm.

Some years ago lasers with very narrow band widths were developed. Often, the relatively high energy of the focused source of light causes degradation of irradiated substances, and beyond that, the wavelength of light emitted by the lasers can only be varied in a small region of the electromagnetic spectrum (approximately 50–100 nm).

2.1.2 Low-Temperature Spectroscopy

At low temperatures, the interchange of energy between neighboring molecules is reduced. Absorption bands of some materials measured at liquid air temperature are sharpened to approximate more closely the absorption bands of a vapor. In addition to this phenomenon, the absorption-band intensity may be increased by cooling the specimen until it becomes embedded in a mass of microcrystals of ice or other cooling medium. This is useful when observing weak bands. The increase in intensity is due to the larger path of the light passing through the absorbing media which is reflected multiply by the microcrystals [1]. If the band widths are diminished at low temperatures, there is a much greater chance of finding nonoverlapping bands suitable for analysis.

Derivative Spectrophotometry. By Gerhard Talsky

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For practical measurements, special equipment is necessary to cool the substances to low temperatures [2–4]. A small Dewar vessel can be fitted with two plan-parallel silica disks. A stream of dry and cool nitrogen will prevent clouding of the optical windows. Another effective precaution is to use fiber optics, and set the space between the optical windows and the fiber cables under vacuum [3]. Mainly liquid nitrogen and liquid helium are used as cooling agents, or solvents that form a glasslike solid (e.g., ethanol, propanol, methylpentane [4]).

2.1.3 Computation Methods

2.1.3.1 Curve-Fitting Method

The method giving the closest approximation of visual-peak recognition is the *curve-fitting method.* But this requires a clearly known profile function.

The highest peaks are sought first. Next, the theoretical curve of the main components is computet with the aid of the profile function. Then, an additional peak is introduced to the points where the least-squares deviations fail to meet a certain limit. The computation is continued as long as the sum of the least squares is sufficiently small.

The profile and the number of peaks must be precise in order to avoid great uncertainties. Overlapping of signals may alter the number of peaks determined, depending on the shape, height, and width of each of the peaks [5]. For further information, see [5-17].

2.1.3.2 Numerical Multicomponent Analysis

Supposing three analytical peaks are superimposed on each other, and each standard absorption spectrum of the pure substances is known; then, according to the Lambert-Beer law the absorbance A at any wavelength will be the sum of the absorbances of individual component at this wavelength (Fig. 2-1). Mathematically expressed,

$$A_{\lambda_1} = c_A \varepsilon_{\lambda_1,A} + c_B \varepsilon_{\lambda_1,B} + c_C \varepsilon_{\lambda_1,C} \tag{2-1}$$

Three parameters are desired: c_A, c_B, and c_C. Therefore, at least three equations are necessary to solve the problem:

$$A_{\lambda_2} = c_A \varepsilon_{\lambda_2,A} + c_B \varepsilon_{\lambda_2,B} + c_C \varepsilon_{\lambda_2,C} \tag{2-2}$$

and

$$A_{\lambda_3} = c_A \varepsilon_{\lambda_3,A} + c_B \varepsilon_{\lambda_3,B} + c_C \varepsilon_{\lambda_3,C} \tag{2-3}$$

etc.

In practice two ways for multicomponent analysis are common: a) The number of wavelengths measured and the number of equations used are equal the number of substances *(lowest determined system of equations).* b) The number of wavelengths

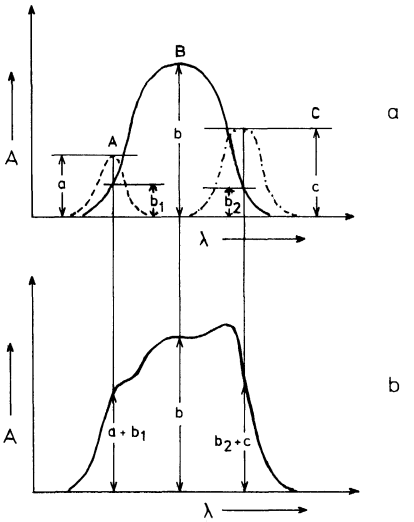

Figure 2-1. Superposition of three analytical peaks. a) Single peaks A (----), B (——), and C (- · - ·); b) Overlapping by summation.

measured and the number of equations used exceeds the number of substances *(over-determined system of equations).*

In both cases, accurate results are only obtained if the most favorable measuring points are chosen, and no unknown substances or undefinable background interference is present [18]. One way to resolve a mixed spectrum more accurately is to operate in the derivative domain; each standard spectrum, along with the mixture, is transformed to the first-, second-, or higher-order derivative. Linear combinations can then be computed [19]. For further information see [20-24].

2.1.3.3 Fourier Analysis

An alternative numerical method for resolving complicated signals is to analyze the frequency spectrum of the curves. The so-called *Fourier transform analysis* (FT analysis) approximates the sum of sine and cosine functions for empirically generated signals. First, 2 *m* points of supports, spaced equidistantly, are chosen (Fig. 2-2). We recommend choosing a multiple of four for 2 *m* and using the values 12, 24, 36, 72, ...

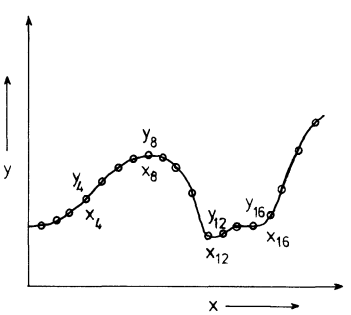

Figure 2-2. Points of supports for Fourier transform analysis of a curve.

Then one can take advantage of the symmetry of the sine and cosine function. The most complex operation in the Fourier expansion,

$$f(x) = a_0 \sum_{n=1}^{\infty} (a_n \cos nx + b_n \sin nx), \qquad n = 1 \text{ to } \infty \tag{2-4}$$

is to calculate the 2 m Fourier coefficients a and b

$$a_n = \frac{1}{m} \sum_{i=0}^{2m-1} y_i \cos \frac{ni}{m} \qquad n = 1, 2, \ldots, m \tag{2-5}$$

$$b_n = \frac{1}{m} \sum_{i=0}^{2m-1} y_i \sin \frac{ni}{m} \qquad n = 1, 2, \ldots, (m-1) \tag{2-6}$$

These algorithms require an enormous database and take relatively long to compute. Smoothing effects can be obtained when the higher frequencies are neglected. Further information is available in special mathematical textbooks and in [10, 25-27].

2.1.3.4 Differentiation

Multi differentiation of electric signals is a powerful method for finely resolving spectra, sharpening peaks, and eliminating unwanted background interference. This technique is either applied directly to the signals or after the curve is fitted with numeric algorithms (cf. 2.1.3.1-2.1.3.3). This method will be treated in detail in this monograph.

2.2 Differentiation and Derivative Spectra

The differentiation of a curve or of its mathematical function is simply an estimation of the slope over the whole region. In the same way, it is possible to differentiate a spectrum (Fig. 2-3). In spectroscopy, the measured value is the ratio of the intensity of the

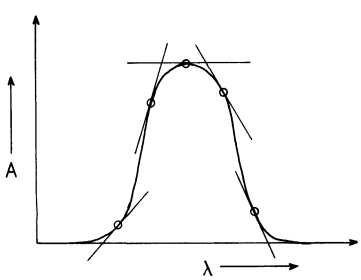

Figure 2-3. Tangents of a curve at defined points (schematic drawing).

light after it leaves the sample (I) to the intensity of the light before it enters the sample (I_0). This ratio is the *transmittance T:*

$$\frac{I}{I_0} = T \tag{2-7}$$

The decrease in intensity of the light passing through a homogeneous transparent sample is not linear, as shown in Fig. 2-4.

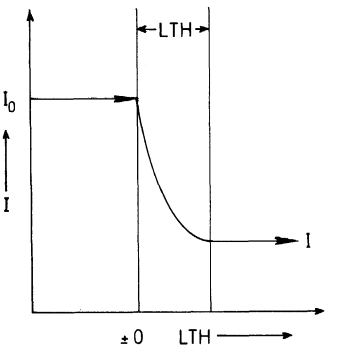

Figure 2-4. Decrease in intensity of light passing through a sample. LTH: layer thickness (= pathlength l); I: intensity; I_0: intensity of the original light (schematic drawing).

The relation between I and the concentration of either a dissolved sample, a transparent liquid, or a solid is given by *Bougueur-Lambert-Beer* law:

$$\frac{I_\lambda}{I_{0,\lambda}} = e^{-cl\varepsilon_\lambda} = T_\lambda \tag{2-8}$$

c: concentration of the absorbing species (mol/L)
l: pathlength (cm)
ε_λ: molar absorption, coefficient of absorbing species at wavelength λ
T_λ: transmittance at wavelength λ.

Usually the intensity I_0 is held constant over the entire range of wavelengths with an automatically controlled slit, automatic electronic adjustment of amplification, or automatic control of the voltage of the light source.

Taking the natural logarithm of Eq. 2-8 gives

$$\ln I_\lambda - I_{0,\lambda} = -cl\varepsilon_\lambda \tag{2-9}$$

This expression is now differentiated:

$$\frac{\mathrm{d}(\ln I)}{\mathrm{d}\lambda} = -cl\frac{\mathrm{d}\varepsilon}{\mathrm{d}\lambda} \tag{2-10}$$

If I_0 is constant, then

$$\frac{d(\ln I_0)}{d\lambda} = 0 \tag{2-11}$$

and

$$\frac{d \ln I}{d\lambda} = \frac{1}{I} \tag{2-12}$$

The first derivative of Eq. (2-8) is obtained as

$$\frac{dI}{d\lambda} \cdot \frac{1}{I} = -cl \frac{d\varepsilon}{d\lambda} \tag{2-13}$$

The first derivative is directly proportional to the concentration at each wavelength. The sensitivity of the measurement is particularly high near points of inflection, where the values of $d\varepsilon/d\lambda$ are extreme.

For the second derivative we find:

$$\frac{d^2I}{d\lambda^2} \cdot \frac{1}{I} = c^2 l^2 \left(\frac{d\varepsilon}{d\lambda}\right)^2 - cl \frac{d^2\varepsilon}{d\lambda^2} \tag{2-14}$$

If the first derivative of ε (i.e., $d\varepsilon/d\lambda$) is equal to zero, the second derivative will be directly (linearly) proportional to the concentration. Otherwise no linear relation is found, and a special calibration curve will be necessary. In addition, for extreme values of $d^2\varepsilon/d\lambda^2$, the sensitivity of the measurement is particularly high.

The same holds true for the third derivation,

$$\frac{d^3I}{d\lambda^3} \cdot \frac{1}{I} = -cd \frac{d^3\varepsilon}{d\lambda^3} + 3c^3d^2 \frac{d\varepsilon}{d\lambda} \frac{d^2\varepsilon}{d\lambda^2} - c^3d^3 \left(\frac{d\varepsilon}{d\lambda}\right)^3 \tag{2-15}$$

In this case, $d\varepsilon/d\lambda$ must also be zero for the third derivative to be linearly proportional to the concentration. Particularly high measurement sensitivity is obtained at shoulders with horizontal tangents to the points of inflection and small radii of curvature. If, however, $d\varepsilon/d\lambda$ is not zero (i.e., if one is dealing with points without horizontal tangents), then nonlinear calibration curves must be prepared.

For the fourth derivative, it follows that

$$\frac{d^4I}{d\lambda^4} \cdot \frac{1}{I} = -cd \frac{d^4\varepsilon}{d\lambda^4} + 4c^2d^2 \frac{d\varepsilon}{d\lambda} \frac{d^3\varepsilon}{d\lambda^3} + 3c^2d^2 \left(\frac{d^2\varepsilon}{d\lambda^2}\right)^2 - 6c^3d^3 \frac{d^2\varepsilon}{d\lambda^2} \left(\frac{d\varepsilon}{d\lambda}\right)^2 + c^4d^4 \left(\frac{d\varepsilon}{d\lambda}\right)^4 \tag{2-16}$$

Linear proportionality is given only when both $d\varepsilon/d\lambda$ and $d^2\varepsilon/d\lambda^2$ assume values of zero. For this reason the derivatives of transmittance are rarely used, although transmittance is the physical quantity that can be directly measured in spectrophotometry. All other quantities, for example absorbance A, $\log A$, or concentration c, are derived quantities. They are computed from the fundamental quantity T.

Equations for even higher derivatives can be set up in a similar way. The above-mentioned absorbance A, is defined as

$$A = \log_{10} I_0/I = \log_{10} \frac{1}{T} = cl\varepsilon' \tag{2-17}$$

ε' is now called the *molar extinction coefficient* of the absorbing solution. It follows that

$$\varepsilon' = \varepsilon/2.303 \tag{2-18}$$

If I_0 is held constant, then for the first derivative,

$$\frac{dA}{d\lambda} = cl\frac{d\varepsilon'}{d\lambda} \tag{2-19}$$

and for the second,

$$\frac{d^2A}{d\lambda^2} = cl\frac{d^2\varepsilon'}{d\lambda^2} \tag{2-20}$$

For the nth-order derivative,

$$\frac{d^nA}{d\lambda^n} = cl\frac{d^n\varepsilon'}{d\lambda^n} \tag{2-21}$$

In all cases, A as well as all derivatives of A are linearly proportional to the concentration c. This is a great advantage in computation and interpretation of derivative spectra, and thus in practice, derivatives of A are commonly generated, while derivatives of T are rare.

Sometimes differentiation of A may result in nonlinear dependence of the derivatives on the concentration. This is always the case if Beer's law is not obeyed, for example, if interactions between the molecules of the substance or between the substance and the solvent occur, or if association and dissociation are observed. Also, it may be that so-called satellites are disturbing main signals; in this case, a higher or lower order of differentiation must be calculated.

2.3 Derivatives of Analytical Bands

An absorption band, also called an analytical band, can be more accurately described by approximation formulas. Gaussian functions are well suited for describing UV-VIS bands [28–30]. The absorbance A of the band at wavelength λ is given by

$$A_\lambda = A_{max}\, e^{-Cx^2} \tag{2-22}$$

A_{max} absorbance at λ_{max}
C: constant
x: $(\lambda - \lambda_{max})$

Differentiating this equation with respect to x leads to the following expressions:

$$\frac{dA_\lambda}{d\lambda} = d^1 = (-2)\, Cx \cdot A_\lambda \tag{2-23}$$

$$\frac{d^2A_\lambda}{d\lambda^2} = d^2 = 2C(2Cx^2 - 1) \cdot A_\lambda \tag{2-24}$$

$$\frac{d^3A_\lambda}{d\lambda^3} = d^3 = (-4)C^2x(2Cx^2 - 3) \cdot A_\lambda \tag{2-25}$$

$$\frac{d^4A_\lambda}{d\lambda^4} = d^4 = 4C^2(4C^2x^2 - 12Cx^2 + 3) \cdot A_\lambda \tag{2-26}$$

$$\frac{d^5A_\lambda}{d\lambda^5} = d^5 = (-8)C^3x(4C^2x^4 - 20Cx^2 + 15) \cdot A_\lambda \tag{2-27}$$

$$\frac{d^6A_\lambda}{d\lambda^6} = d^6 = 8C^3(8C^3x^6 - 60C^2x^4 + 90Cx^2 - 15) \cdot A_\lambda \tag{2-28}$$

Other symbols for the derivatives are

A, A', A'', A''', A'''', ...
A^0, A^I, A^{II}, A^{III}, A^{IV}, ...
Abs., 1st Der, 2nd Der, 3rd Der, ...
$D0$, $D1$, $D2$, $D3$, $D4$ ...
A, $1D$, $2D$, $3D$, $4D$ ...

The last two alternatives are especially common in computer printouts.
For the constant C, the *half width* of the band is taken into consideration. The following two definitions are used for the half width (Fig. 2-5):

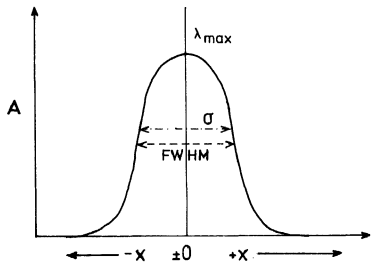

Figure 2-5. Schematic drawing of FWHM and σ.

a) *FWHM* (full width at half maximum) amplitude, the width of the band at half the height of the maximum;

b) σ, the width of the band between the points of inflection.

For an analytical band positioned symmetrically to the right and left of the coordinate origin (Fig. 2-5), the result of the condition for an inflection point when

$$d^1 = d^2 = 0 \tag{2-29}$$

is

$$x_\sigma = \sqrt{\frac{1}{2\,C}} \tag{2-30}$$

and for the point FWHM, if $A_\lambda = 0.5 \cdot A_{\lambda_{max}}$, then

$$x_{FWHM} = \sqrt{\frac{\ln 2}{C}} \tag{2-31}$$

For the constant C with $x_{FWHM} = 0.5 \cdot \text{FWHM}$ and $x_\sigma = 0.5\ \sigma$ it follows that

$$C_{FWHM} = \frac{4 \ln 2}{(\text{FWHM})} \quad \text{and} \tag{2-32}$$

$$C_\sigma = \frac{2}{\sigma^2} \tag{2-33}$$

FWHM is always greater than σ:

$$\frac{\text{FWHM}}{\sigma} = \sqrt{2 \cdot \ln 2} = 1.177 \tag{2-34}$$

Equations (1-23) to (2-28) show that the Gaussian term A_λ is the same for all derivatives, only the polynomial is modified by differentiation. While the Gaussian distribution function satisfactorily describes the shape of electronic spectra in the UV and visible range [28–30], the Lorentzian distribution function is better qualified for spectra such as IR [31] and Raman [32], etc. For analytical bands in the NIR (near infrared) range, a mixture of the Gaussian and Lorentzian function is recommended [14].

To estimate the absorbance of an analytical band, it is best to take the area under the curve (Fig. 2-6) and integrate numerically, with the help of a mechanical integrator, either by counting the squares of a framed paper or by weighing the paper of the peak after it has been cut out. The product of the peak height A at λ_{max} ($A_{\lambda_{max}}$) and the halfwidth σ is a good approximation of the area if the bands are symmetrical. However, in practical spectrophotometry it is sufficient to let $A_{\lambda_{max}}$ be proportional to the concentration of the substance.

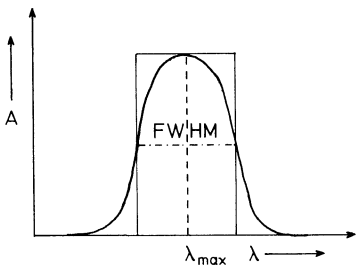

Figure 2-6. An "analytical peak". The area below the curve is approximately equal to the area of the rectangle (product of λ_{max} and FWHM or σ, depending on the type of curve function; qualitative graph).

The results of the differentiation of simple analytical bands are represented graphically in Fig. 2-7. From these results (and from [33]) we learn that:

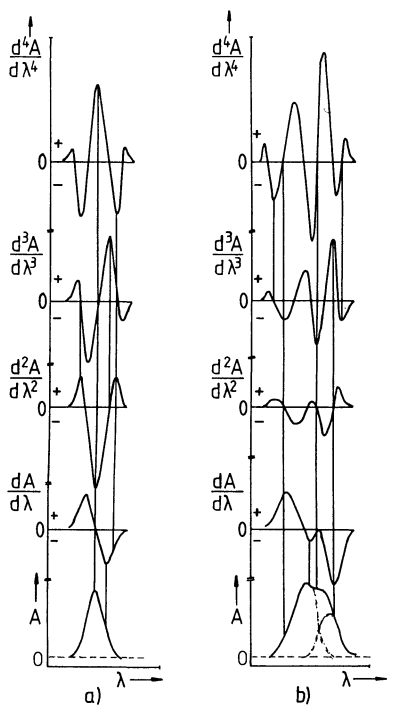

Figure 2-7. Differentiation of computed Gaussian analytical bands.
a) Fundamental curve and its first- to fourth-order derivatives; b) Fundamental curve and first- to fourth-order derivatives of two superposed Gaussian bands. The overlapping peaks become separated by this manipulation.

a) The maximum of the fundamental curve in odd-order derivatives corresponds to a passage through zero, and in even-order derivatives it corresponds to an extremum value of either a minimum or a maximum. In other words, the basic maximum always produces a positive extremum in the 4th, 8th, and 4*n*th derivative, and a negative extremum (minimum) in the 2nd, 6th, and (4*n*−2) th derivative (*n* = 1, 2, 3 ... in both cases).

b) Inflections on the fundamental curve lead to odd-order extrema and to passages through zero in even-order derivatives.

c) With increasing order of the derivative, the number of extrema exceeds that of the fundamental curve because each inflection gives an additional extremum by differentiation. In this case one speaks of *virtual extrema,* or *satellites.* In the *n*th derivative, one maximum produces *n* + 1 extrema. In other words, besides one main extremum there are *n* smaller satellite bands.

d) With increasing derivative order the sharpness of the bands increases – and σ, as well as FWHM, becomes smaller.

The direction of the differentiation determines the sign of the function. A positive slope of the curve always gives a positive value of the derivative and a negative slope results in a negative value of the derivative. In Fig. 2-8 the direction is from left to right; if differentiation proceeds from right to left, then the curves are inverted. Statements c) and d) are especially important and thus need to be discussed in more detail.

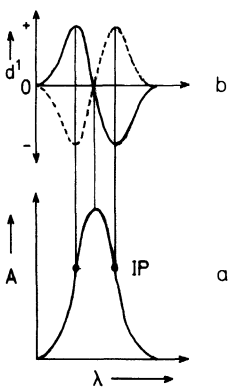

Figure 2-8. Influence of the direction of differentiation on the sign of the function.
a) Fundamental curve; b) first derivative (d^1), taken from left to right side (——) and right to left side (- - - -); IP: inflection point (qualitative graph).

2.3.1 Virtual Extrema (Satellites)

With virtual extrema, inflections are more readily determined, and therefore the spectrum is easier to resolve. On the other hand, with increasing derivative order, simplicity is lost and satellites may sometimes superpose extrema of neighboring analytical peaks. For this reason, with increasing differentiation order, a maximum amount of information will be run through, depending on the complexity of the signals.

Fortunately, the ratio (R_n) of the satellite height S_n to the height of the main peak A_0 does not increase linearly. In d^2, the value for R_1 is 45%, in d^{10} it is not approximately 120% (extrapolated) but only 80% (Fig. 2-9a and 2-9b) [34, 35].

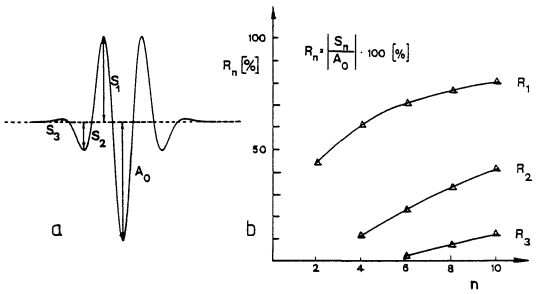

Figure 2-9. Influence of derivative order on the height of satellite bands (according to [35]). a) Main peak A_0 and its satellites S_1, S_2, and S_3 of second-order derivative (schematic drawing). A_0 represents only the part of the original signal from the maximum to the inflection point (PZ method). Therefore, for quantitative evaluations, the sum of A_0 and S_1 is usually taken (PP method). b) Dependence of the relative peak amplitude R_n on the derivative order n (real diagram).

Fell [36] studies the satellites of Gaussian and Lorentzian functions of even orders up to d^{10}. He used a more common but also more complex expression of the Gaussian distribution function.

$$A_n = S \cdot \frac{A_{max}}{\sigma^n} \cdot \exp\left(\frac{-z^2}{2\sigma^2}\right) \cdot P, \tag{2-35}$$

and

$$P = b_0 - b_1 x + b_2 x^2 - b_3 x^3 + b_4 x^4 - b_5 x^5$$

n: order of even derivative
S: sign (+ or −)
A_{max}: maximum absorbance at λ_{max} for the zero-order band
x: z^2/σ^2
z: $\lambda - \lambda_{max}$ (units of wavelength)
σ: band half width at point of inflection
b_0, b_1: polynomial coefficients (see [36])

Table 2-1. Polynomial coefficients of Gaussian equation (Eq. (2-35)); after [36].

n	S	b_0	b_1	b_2	b_3	b_4	b_5
0	1	1	0	0	0	0	0
2	−1	1	1	0	0	0	0
4	1	3	6	1	0	0	0
6	−1	15	45	15	1	0	0
8	1	105	420	210	28	1	0
10	−1	945	4725	3150	630	45	1

The Lorentzian distribution function is defined as [36]

$$A_n = \frac{A_{\max} b_0}{m^{n+1} \cdot \sigma^n} [1 - b_1 x + b_2 x^2 - b_3 x^3 + b_4 x^4 - b_5 x^5] \quad (2\text{-}36)$$

x: $z^2/2\sigma^2$
m: $1 + z^2/2\sigma^2$

The other signs are identical to those in Eq. (2-35) (see Table 2-2). By comparing the derivatives of a Gaussian-shaped analytical peak with a Lorentzian peak (Fig. 2-10), it is remarkable that the outlying satellites of the latter interfere less than

Table 2-2. Polynomial coefficient of Lorentzian equation (Eq. (2-36)); after [36].

n	b_0	b_1	b_2	b_3	b_4	b_5
0	1	0	0	0	0	0
2	−1	3	0	0	0	0
4	6	10	5	0	0	0
6	−90	21	35	7	0	0
8	2520	36	126	84	9	0
10	−113400	55	330	462	165	11

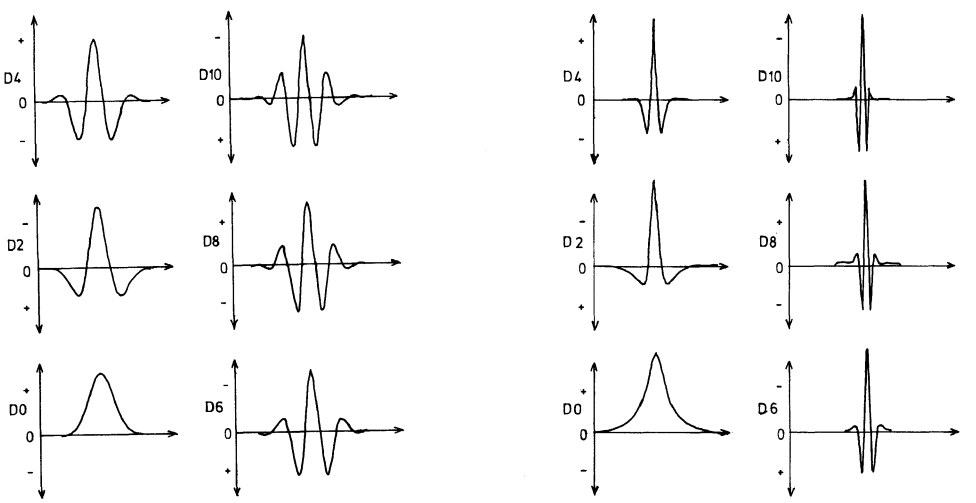

Figure 2-10. Differentiation of curves depending on their shape.
a) Gaussian peak; even derivatives up to the tenth order; b) Lorentzian peak; even derivatives up to the tenth order. (For better comparison, the signs of D2, D6, and D10 are inverted.) The main signals of the Lorentzian type are thinner than those of the Gaussian type. Also, the satellites of the former are smaller. All derivatives are computed by means of Eq. (2-35) and Eq. (2-36) after [36].

those of the Gaussian function. Detailed quantitative calculations up to the 10th order can be found in [36]. Another interesting feature of the satellite patterns is that the outlying Gaussian satellites extend far outwards from the centroid (to about $\pm 3.5\,\sigma$); their Lorentzian equivalents are closer to the centroid and generally remain undetected beyond $\pm 1.5\,\sigma$ [36].

2.3.2 Sharpening of Peaks and Shoulders

As stated in Sec. 2.3, peaks are sharpened by increasing derivative order, as can be seen in Fig. 2-11 a and 2-11 b. In the second derivative, the FWHM has only 53% of the value of the original FWHM (d^0), and d^{10} shows only 26.6%. Figure 2-11 b also demonstrates that derivative orders higher than six contribute only littel to signal sharpening. The smaller the FWHM value, the better the possibility of separating overlapping peaks. This is one of the features of higher-order derivates ($n > 2$).

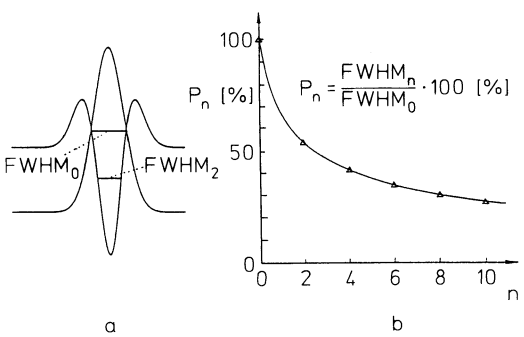

Figure 2-11. Influence of the order of derivatives on FWHM.
a) Relative peak half-width P_n (for a fundamental peak and its second derivative), with P_n reaching 66.6% (schematic drawing); b) FWHM as a function of derivative order n, where P_n varies with the mode of the distribution curve.

Another important effect if peaks with different peak-FWHM ratios are differentiated, made evident in Fig. 2-12, is that flat signals become smooth and steep signals become sharp. It can be shown that e.g. the maximal height of the inverted second derivative is proportional to the second power of the inverted ratio

$$A^{II} = -\frac{A^0}{(\mathrm{FWHM})^2} \tag{2-37}$$

and, if $A_1^0 = A_2^0$, and $\mathrm{FWHM}_1 > \mathrm{FWHM}_2$, then

$$\frac{A_1^{II}}{A_2^{II}} = \left(\frac{\mathrm{FWHM}_2}{\mathrm{FWHM}_1}\right)^2 \tag{2-38}$$

Derivative peaks of n^{th} order need n^{th} power of the FWHM ratio in Eq. (2–38)

In this way small shoulders are strengthened, but the main signal, which is flat, disappears with higher-order differentiation (see Fig. 2-13). Signal sharpening of shoulders also becomes evident in Fig. 2-14 [33]. For further information see [33 and 36–40].

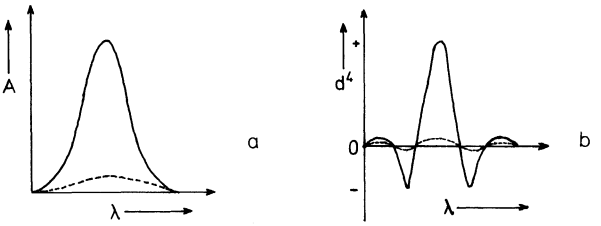

Figure 2-12. Suppression of flat signals and sharpening of steep peaks.
a) Fundamental spectra;
b) fourth-order derivatives spectra (after [37], qualitative graphs).

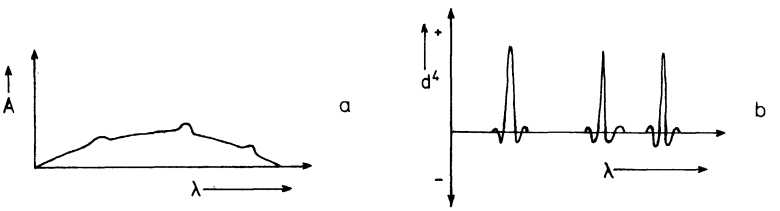

Figure 2-13. Elimination of flat signals.
a) Fundamental spectrum; b) fourth derivative of a) (synthetic spectra).

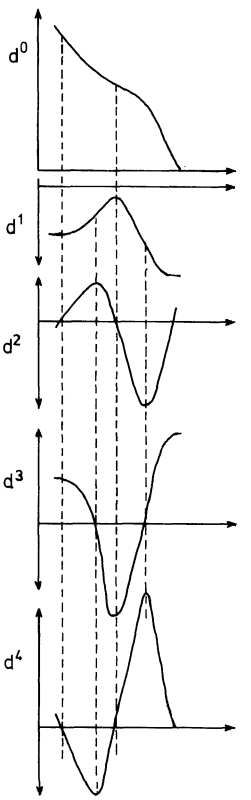

Figure 2-14. Signal sharpening by differentiation of shoulders. Fundamental curve and the first to fourth derivative (after [33]). In this case, the inflection point in the odd derivatives always gives an extremum, and in even derivatives, a zero crossing.

2.3.3 Overlapping of Signals

Differentiation of simple analytical peaks is recommended whenever signals are superposed by background, as caused by turbid solutions or by opaque solids.

The first application of differentiation to spectroscopy was, of course, the deconvolution of superposed peaks [41–43], which are frequently found in spectral investigations. From then on, many scientists analyzed peak overlapping by summation of synthetic signals, mostly of the Gaussian type [12, 14, 15, 44–48]. Derivatives, zero crossing, and extrema of different distribution functions such as Gauss, Lorentz, Student, T_3, and others, are not difficult to estimate unless there are superpositions of two or more bands. For pure Gaussian functions, see Table 2-3.

Table 2-3. Extrema and zero crossing for Gaussian functions and their derivatives (d^1 to d^4); after [46].

	Function[a)]
d^0	$y_0 \exp[-(Du^2/2)]$
Extrema	y_0, x_0
Zero crossing	–
d^1	$-Dy_0 u \exp[-(Du^2/2)]$
Extrema	$\pm 1.428\, y_0/W$, $x_0 \pm 0.4247\, W$
Zero crossing	x_0
d^2	$Dy_0(Du^2-1) \exp[-(Du^2/2)]$
Extrema	$-5.545\, y_0/W^2$, x_0; $2.474\, y_0/W^2$, $x_0 \pm 0.7355\, W$
Zero crossing	$x_0 \pm 0.4247\, W$
d^3	$-D^2y_0u(Du^2-3) \exp[-(Du^2/2)]$
Extrema	$\pm 18.020\, y_0/W^2$, $x_0 \pm 0.3150\, W$;
	$\pm 4.895\, y_0/W^2$, $x_0 \pm 0.9914\, W$
Zero crossing	x_0, $x_0 \pm 0.7355\, W$
d^4	$D^2y_0(D^2u^4-6Du^2+3) \exp[-(Du^2/2)]$
Extrema	$92.24\, y_0/W^4$, x_0; $-57.0317\, y_0/W^4$, $x_0 \pm 0.575\, W$;
	$10.722\, y_0/W^4$, $x_0 \pm 1.2132\, W$
Zero crossing	$x_0 \pm 0.3150\, W$, $x_0 \pm 0.9914\, W$

[a)] *u:* $x-x_0$; *W:* FWHM; *D:* $5.545/W^2$; y_0: absorption at maximum (x_0); functions are symmetrical, with $-x$ and $+x$ to the left and right of x_0.

Some examples of peak overlapping are shown in Fig. 2-15. Two Gaussian peaks were added whose maxima do not have the same position on the abscissa. Even if the superposition of these two signals leads to two well-separated peaks, the positions of the maxima may not coincide with those of the original peaks. The positions will correspond only if the distance between the maxima is broad enough (Fig. 2-15 b). This will be treated quantitatively in Sec. 2.3.4.

Whereas a mould was formed between the two maxima in Fig. 2-15a, only a symmetrical peak with one maximum can be seen in Fig. 2-15c. Two peaks of different height give a main peak with a *shoulder* (Fig. 2-15d) or, if the original maxima are nearby and the distance between them is smaller than the greatest FWHM, only a distorted, unsymmetrical signal is developed (Fig. 2-15f). The point at which the two peaks overlap

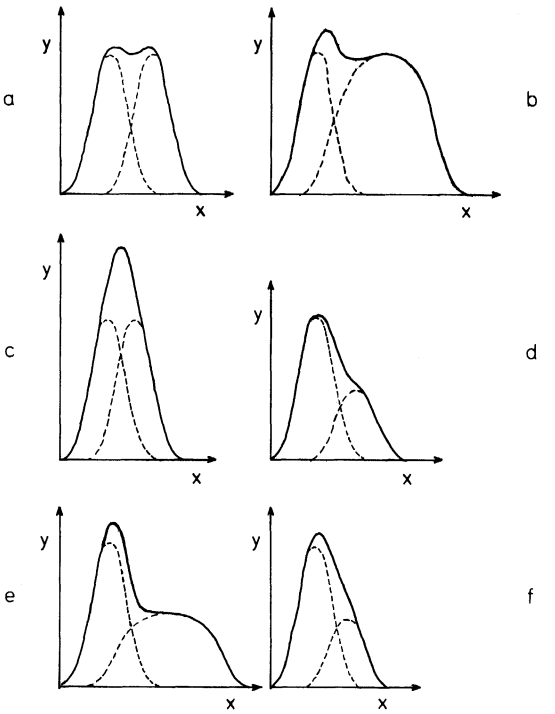

Figure 2-15. Some examples of peak overlapping. In a, b, and c the peak heights of the original maxima are identical. If the distance between the original maxima is smaller than the half width, only one peak results (c and f).

to the degree that the valley between them just disappears is generally defined as the *shoulder limit* for the peaks [11]. If the distances are smaller than this critical distance, d_c, one of the bands becomes a shoulder without a maximum and the original maximum corresponds to an inflection point in the superposed curve. Then

$$\frac{dy}{dx} = \frac{d^2y}{dx^2} = 0 \tag{2-39}$$

d_c depends on the ratio of the amplitudes ($R = A_2/A_1$) and also on the ratio of the half width ($\varphi = \mathrm{FWHM}_1/\mathrm{FWHM}_2$). If R and φ are known, it is possible to make a prognosis for d_c [8, 15]. Generally this value decreases when the order of differentiation increases [15].

Supposing two Gaussian peaks have the same amplitude ($A_1 = A_2$) and the same half width ($\mathrm{FWHM}_1 = \mathrm{FWHM}_2$), then the shoulder limit is dependent on the

derivative order (Fig. 2-16). Along the dotted line (45 ° angle), t_s is equal to t. If the two peaks move even closer together, the result is only a single signal, whereby one of its flanks is distorted. This critical distance, when the inflection point of the smaller peak disappears, is called the *detection limit* [8]. Then

$$\frac{d^2y}{dx^2} = \frac{d^3y}{dx^3} = 0 \tag{2-40}$$

Figure 2-16 also shows that with higher-order derivatives, the detection limit moves to smaller values of t, which is attributed to the sharpening of the signals.

Two peaks with identical maxima with respect to λ cannot be separated even if the half widths are different. Higher-order differentiation may only cause the signal with the larger FWHM to be eliminated. Nevertheless, it is worthwhile to at least try to shift the maxima, either by changing the pH or by altering the solvent.

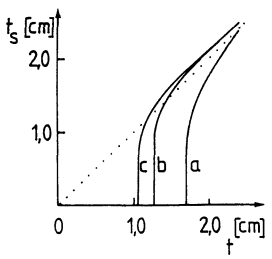

Figure 2-16. Shoulder limit plotted as a function of the derivative order. $A_1 = A_2 = 100$ mm; $FWHM_1 = FWHM_2 = 16.65$ mm; t_s: distance between the maxima in the overlapping peaks; t: distance between the maxima of the two single peaks (according to [15]); a, b, and c correspond to d^0, d^2, and d^4, respectively.

2.3.4 Shifting of Maxima

In quantitative investigations only the amplitude of the derivative signal is relevant for the concentration of a substance; however, the shifting of extrema may be important whenever the original position of each superposed peak is to be found. In Sec. 2.3.3 it was mentioned that the extrema of the overlapping peaks only coincide with the original maxima of the single signals if the distance separating them has a minimum value, which depends on their height and half width.

For a quantitative estimate of deviations, different spectra of two overlapping Gaussian peaks were simulated by computer, and the derivatives were generated. The following expressions for a numerical description of fundamental and derivative spectra (d^0 to d^6) were used:

$$S^0 = A_1^0 + A_2^0 = A_{max1}\, e^{-C_1 x^2} + A_{max2}\, e^{-C_2(x-t)^2} \tag{2-41}$$

$$S^I = (-2)C_1 x \cdot A_1^0 + (-2)C_2(x-t) \cdot A_2^0 \tag{2-42}$$

$$S^{II} = 2C_1(2C_1x^2 - 1) \cdot A_1^0 + 2C_2(2C_2(x-t)^2 - 1) \cdot A_2^0 \tag{2-43}$$

$$S^{III} = (-4)C_1^2 x(2C_1x^2 - 3) \cdot A_1^0 + \\ + (-4)C_2^2(x-t)(2C_2(x-t)^2 - 3) \cdot A_2^0 \tag{2-44}$$

$$S^{IV} = 4C_1^2(4C_1^2x^4 - 12C_1x^2 + 3) \cdot A_1^0 + \\ +4C_2^2(4C_2^2(x-t)^4 - 12C_2(x-t)^2 + 3) \cdot A_2^0 \quad (2\text{-}45)$$

$$S^{V} = (-8)C_1^3x(4C_1^2x^4 - 20C_1x^2 + 15) \cdot A_1^0 + \\ + (-8)C_2^3(x-t)(4C_2^2(x-t)^4 - 20C(x-t)^2 + 15) \cdot A_2^0 \quad (2\text{-}46)$$

$$S^{VI} = 8C_1^3(8C_1^3x^6 - 60C_1^2x^4 + 90C_1x^2 - 15) \cdot A_1^0 + \\ +8C_2^3(8C_2^3(x-t)^6 - 60C_2^2(x-t)^4 + 90C_2(x-t)^2 - 15) \cdot A_2^0 \quad (2\text{-}47)$$

t: distance of single peaks in the fundamental curve
C: constant

Varying the amplitudes and FWHM of the peaks beginning with their distance t allows each superposition to be generated. The positions of the maxima and minima were determined by a computer program. An extremum was reached when there was a sign change for the difference of two neighboring amplitudes.

Two counter-rotating effects are evident. First, with increasing derivative order the overlapping of satellites increases and may cause shifting of extrema; and second, peaks are sharpened and overlapping decreases. Therefore, an exact and general prognosis for peak shifting is not possible.

Recently the shifting of maxima by superposition of computed Gaussian peaks was simulated [34, 35], and their behavior was studied.

Three examples demonstrate the superposed signals and the deviations of the extrema's apparent positions from the original positions of the single nonoverlapping peaks (Fig. 2-17, 2-18 and 2-19): the shifts are plotted against the original distance between the maxima. A shift to the left is indicated by a minus sign, and a shift to the right, by a plus sign. Subscript 1 refers to the left peak (A_1, $FWHM_1$, P_1), subscript 2 to the

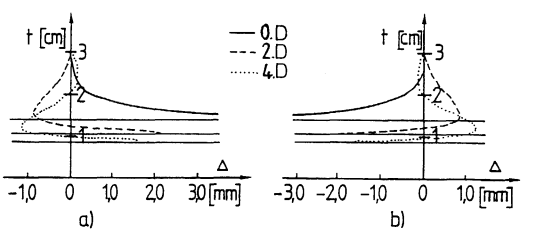

Figure 2-17. Shift (Δ) in the positions of the single peaks by overlapping of two Gaussian bands at distance t for d^0 (0.D), d^2 (2.D), and d^4 (4.D), computed according to Eq. (2-41) to Eq. (2-47). $A_1 : A_2 = 1$; $FWHM_1 : FWHM_2 = 1$. The lines parallel to the x-axes are the "detection limits"; a left peak, b right peak.

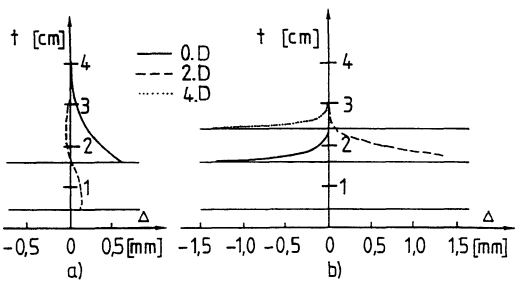

Figure 2-18. Shift (Δ) in the positions of the single peaks by overlapping of two Gaussian bands at distance t. $A_1 : A_2 = 2$; $FWHM_1 : FWHM_2 = 0.5$; a left peak, b right peak.

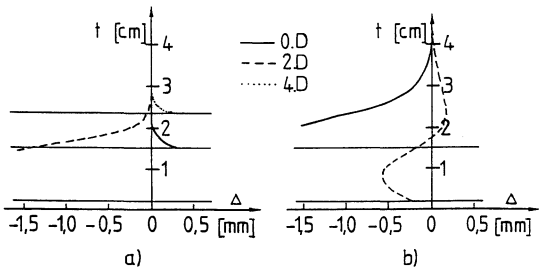

Figure 2-19. Shift (Δ) in the positions of the single peaks by overlapping of two Gaussian bands at distance t. $A_1 : A_2 = 2$; $FWHM_1 : FWHM_2 = 2$; a left peak, b right peak.

right peak. First, in Table 2-4, and in Fig. 2-17a and b, there are two symmetrical Gaussian functions with their maxima separated by a distance t. Both amplitudes (A_1, A_2) are 100 mm and both half widths ($FWHM_1$, $FWHM_2$) are 16.65 mm. For d^0, d^2, and d^4, the detection limits, as well as the maximal and zero shifts were computed – the latter as a shift of 10^{-4} mm. The two graphs are symmetrical because we have two identical signals. In the fundamental curve (d^0) there is only a shift of the maxima toward each other. The greatest deviations (Δ) are near the detection limit ($\Delta > 3$ mm). In d^2 and d^4 the shifts are smaller and, what is more important, they are in both directions. In the second example (Table 2-5 and Fig. 2-18) the amplitudes as well

Table 2-4. Shift of maxima positions by overlapping of two identical Gaussian bands (in mm).[a]

	d^0		d^2		d^4	
	P_1	P_2	P_1	P_2	P_1	P_2
Detection limit	14.14	14.14	10.50	10.50	8.75	8.75
Zero shift	29.69	29.69	32.55	32.55	34.69	34.69
Maximum shift[b]	$> +3.0$	> -3.0	-0.9	$+0.9$	$\leqslant +0.2$	$\leqslant -0.2$
			$\leqslant +2.0$	$\leqslant -2.0$	$\leqslant -1.25$	$\leqslant +1.25$
					$> +1.5$	> -1.25

[a] $A_1 = A_2 = 100$ mm; $FWHM_1 = FWHM_2 = 16.65$ mm.
[b] For example, $> +3.0$ signifies that the left peak (P_1) shifts more than 3 mm to the right and > -3.0 means that the right peak (P_2) shifts to the left by more than 3.0 mm.

Table 2-5. Shift of maxima positions by overlapping of two Gaussian bands of different height and different FWHM (in mm).[a]

	d^0		d^2		d^4	
	P_1	P_2	P_1	P_2	P_1	P_2
Detection limit	16.26	16.26	4.53	4.53	24.26	24.26
Zero shift	37.03	22.91	36.45	26.65	24.87	29.71
Maximum shift[b]	$< +0.6$	< -1.4	-0.03	$> +14.8$	± 0	< -1.5
			$+0.06$			

[a] A: $A_2 = 2.0$; $FWHM_1$: $FWHM_2 = 0.5$.
[b] see footnote in Table 2-4.

as the halfwidth are A_1:100 mm; A_2:50 mm; A_1/A_2:2.0; $FWHM_1$:11.77 mm; $FWHM_2$:23.55 mm; and $FWHM_1/FWHM_2$:0.5. Table 2-5 lists the calculated detection limit, zero shift and maximum shift. In the third example (Fig. 2-19), the $FWHM_1$ and $FWHM_2$ of the foregoing case are inverted: A_1:100 mm; A_2:50 mm; A_1/A_2:2.0; $FWHM_1$:23.55 mm; $FWHM_2$:11.77 mm; and $FWHM_1/FWHM_2$:2.0. The corresponding data can be found in Table 2-6. Further examples can be found in [49] and [50]. Summarizing, if two peaks overlap, then: a) in d^2 and d^4, the broader signal is shifted further; b) in d^0, d^2, and d^4, near the detection limit shifting is strongest; and c) the higher the even derivative order, the smaller the shifting of the extrema of the signals.

As long as the ratio of the peak height to the half width is smaller than about 5 and the distance of the two peak maxima not too near the detection limit, the shift of the signal is generally not greater than a few percent of the peak amplitude. Therefore, it is preferable to use peak sharpening, especially for the higher-order derivatives, so that the signals become separated or, at least, less superposed. In such cases, the deviations do not exist or are small enough to be neglected.

Table 2-6. Shift of maxima positions by overlapping of two Gaussian bands of different height and different FWHM (in mm).[a)]

	d^0		d^2		d^4	
	P_1	P_2	P_1	P_2	P_1	P_2
Detection limit	15.30	15.30	1.97	1.97	12.23	12.23
Zero shift	21.51	40.71	25.06	41.18	28.27	27.39
Maximum shift[b)]	$< +0.4$	< -5.0	> -14.6	$< +0.2$ < -0.6	$< +0.3$	± 0

[a)] $A_1:A_2 = 2.0$; $FWHM_1:FWHM_2 = 2.0$

[b)] see footnote in Table 2-4

2.4 Loss of Information vs. Increase in Resolution by Differentiation

The differentiation of signals can never result in more information than is present in the original bands – as determined by spectrophotometer specifications – but it is possible to bring desired information to the fore and to eliminate unwanted background. This can be compared to a photographer who takes a picture using a normal objective. The main object as well as the surroundings are sharp. But if prominence is to be given to the object, a telephoto lens is used. Then the object becomes sharp and enlarged, and can be seen in detail, but the background is more or less out of focus. Unwanted information is lost in favor of the main object.

Distribution functions (e.g., Gaussian or Lorentzian distributions) are e functions; their differentiation leads to further e functions, which are extended by only one factor, see Eqs. (2-23)–(2-28) in Sec. 2.3. Although these distribution equations become

more complex by differentiation, a polynomial of the nth power is zero after $(n + 1)$ differentiating steps.

$$U^0 = az^4 + bz^3 + cz^3 + dz + e \tag{2-48}$$

$$U^{\mathrm{I}} = 4az^3 + 3bz^2 + 2cz + d \tag{2-49}$$

$$U^{\mathrm{II}} = 12az^2 + 6bz + 2c \tag{2-50}$$

$$U^{\mathrm{III}} = 24az + 6b \tag{2-51}$$

$$U^{\mathrm{IV}} = 24a \text{ (a constant, parallel to the } x\text{-axis)} \tag{2-52}$$

$$U^{\mathrm{V}} = 0 \tag{2-53}$$

Extending Eq. (2-23) to Eq. (2-28) with the polynomials U^0 to U^{n}, we have

$$D^0 = d^0 + U^0 \tag{2-54}$$

$$D^1 = d^1 + U^1 \tag{2-55}$$

and

$$D^n = d^n + U^n \tag{2-56}$$

If U^n is zero, then

$$D^n = d^n + 0 = d^n \tag{2-57}$$

It is therefore possible to eliminate disturbing background absorption (e.g., in turbid solutions or opaque samples) caused by stray light (Fig. 2-20).

Differentiation can thus considerably improve the detection sensitivity of masked weaker bands; for the first time, a qualitative or quantitative determination of these bands is now possible.

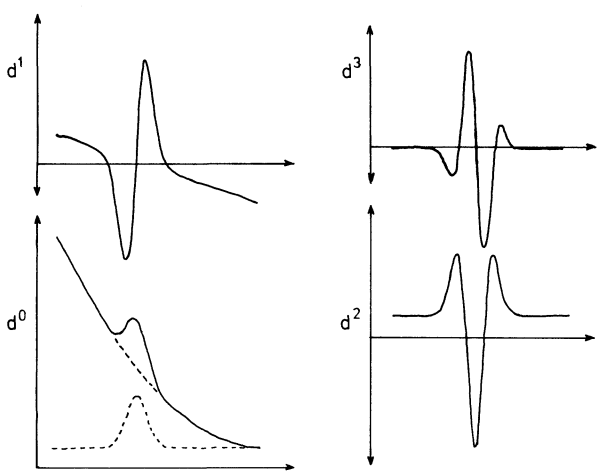

Figure 2-20. Differentiation of a Gaussian band, superposed by a background signal (d^0 to d^3). The disturbing background was eliminated by threefold differentiation.

2.5 "Real" Spectra and Noise

In contrast to digitally generated bands, "real" spectra – signals received from a spectrophotometer – are composed of the actual signal, caused by absorbance of the substance, and noise (Fig. 2-21 a). *Noise* is the generally higher-frequency variation of the

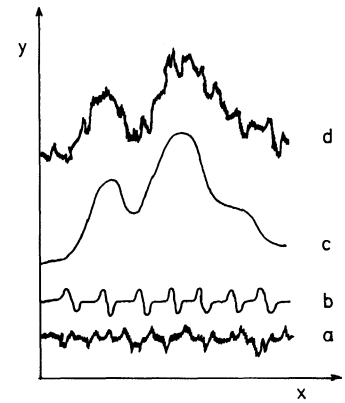

Figure 2-21 a. Superposition of effective and interfering signals. a: Aperiodic (random) interference, b: periodic interference, c: effective signal, d: superposition of a–c (qualitative graph).

signal potential around a central value, for instance: a) noise caused by the sample (e. g., due to temperature gradient, inhomogeneities or scattering); b) noise due to fluctuations of the light source; c) electrical noise from the spectrophotometer, mostly from the photomultiplier, rectifiers, and amplifiers; d) noise caused by the differentiator unit (analog differentiation); e) noise from AD and DA converters (digital differentiation), because the continuous electrical signal becomes sequenced in discrete signals and vice versa. These manipulations cause in each case an inaccuracy of ±1 digit (see Fig. 2-21 b). The aforementioned sources of noise produce random interference, or *aperiodic noise, whereas periodic noise* is caused especially by f) the mains hum and its harmonic; and g) the chopper in double-beam spectrophotometers that diverts the light beam alternately through the sample and standard.

Disturbing signals may lead to systematic or statistical errors. A detailed analysis of these errors is given by O'Haver et al. [47], and [51–53]; also see [33] and [54–57]. An important quantity for characterizing the quality of spectrum is the ratio

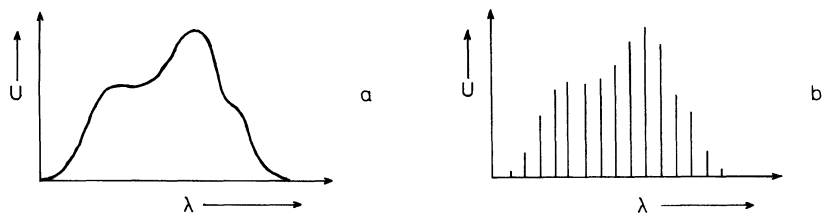

Figure 2-21 b. a) Continuous analog signal; b) discontinuous digital signal. (*U*: voltage).

of the amplitude of the signal (A_S) over the amplitude of the disturbing signal A_{DS}, the *signal-to-noise ratio (SNR)* [53], [58].

$$\mathrm{SNR} = \frac{A_S}{A_{DS}} \tag{2-58}$$

The statement that the SNR decreases with the nth power of two (n = derivative order) [52, 59] is a rule of thumb and is only true for a smoothing ratio r_s of about 0.25 [58]:

$$r_s = \frac{n\,\Delta\lambda}{\mathrm{FWHM}} \tag{2-59}$$

$\Delta\lambda$: distance of data points in digital derivatives
n: number of data points of digital smoothing

Properly speaking, the SNR primarily depends on FWHM [58]:

$$\mathrm{SNR}_n \sim \frac{1}{\mathrm{FWHM}^n} \cdot C \tag{2-60}$$

C: constant; n: derivative order

According to Eq. (2-60) the SNR becomes dramatically smaller with increasing derivative order. This effect becomes intensified in flat bands, where FWHM is high.

The influence of differentiation on noisy spectra is made clear in Fig. 2-22: The SNR already drops from 100 to 2 with the first derivative if no special precautions are

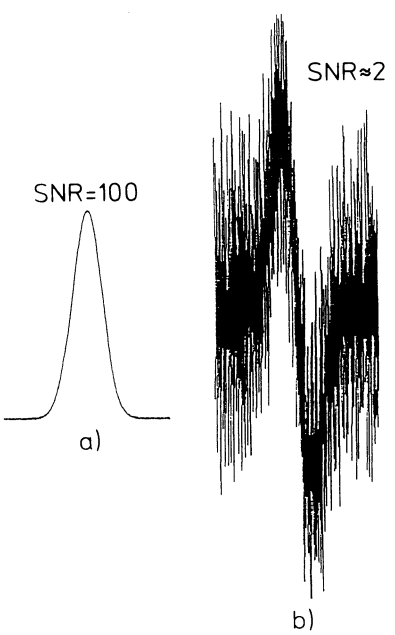

Figure 2-22. Differentiation of an experimental Gaussian signal without lowering of noise.
a) Fundamental signal; b) first derivative. SNR signal-to-noise ratio.

taken [60]. This is also the reason why it has sometimes been said that derivatives of higher order than two are not useful. For the treatment of special precautions, see Sections 3.5.4, 3.6.4 and 4.4.

2.6 Evaluation of Derivative Spectra

2.6.1 Common Methods

Depending on the problem to be solved, different methods of evaluation are used. For quantitative investigations, only the amplitudes of the signals are relevant, and the real position of the extrema are not important if the conditions of data handling are not altered and a calibration curve is taken. But if the exact positions of the extrema are desired to identify peaks in a spectrum or to calculate excitation energies, shifting the maxima and minima must be avoided, or if this is not possible, the deviations must be corrected.

2.6.1.1 Peak-Peak Method

The *peak-peak (PP)* method is most commonly used to estimate concentrations of known substances (Fig. 2-23). In derivatives of absorption curves the distance from a maximum to a minimum is generally directly proportional to the concentration of the substances:

$$\mathrm{d}^n = \frac{\mathrm{d}^n A}{\mathrm{d}\lambda^n} = \frac{d^n \varepsilon}{\mathrm{d}\lambda^n} \cdot c \cdot l \qquad (2\text{-}61)$$

d^n: nth derivative order
A: absorbance
ε: molar absorption coefficient [L mol^{-1} cm^{-1}]
c: molar concentration [mol L^{-1}]
l: cell path length [cm]

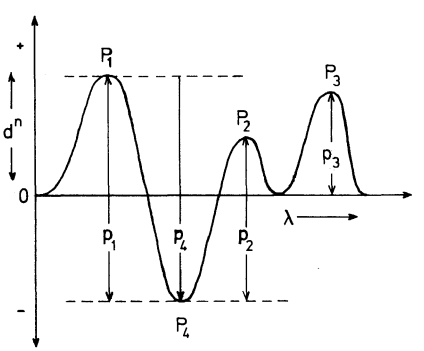

Figure 2-23. Peak-peak evaluation (PP method), where p_n is proportional to the concentration of peak P_n.

In derivatives of the $4n$th order ($n = 1, 2, 3 \ldots$) the positive extrema (P_1, P_2) were evaluated by estimating the distances p_1 and p_2, which are proportional to the concentrations of the relevant substances. In ($4n$-2) derivative orders, p_4 of the negative maximum P_4 has to be measured. This P-P method is frequently used for quantitative multicomponent analysis [33].

If Fig. 2-23 represents a derivative of odd order, P_1 and P_2 are the points of inflection of the fundamental signal, and p_1 and p_2 are proportional to the half width σ. The graph obtained with pure standards is not always a straight line; instead, it can either be a logarithmic, exponential, or some other mathematical function. The main reasons for this are 1) not enough separated peaks; 2) disturbing satellite bands; 3) unwanted background; or 4) the Lambert-Beer law is not obeyed. In these cases, it is necessary to find out whether another peak, or another even or odd order of differentiation, could lead to better results.

Recall that the derivatives of the transmittance are only directly proportional to the concentration under special conditions (see Section 2.2).

2.6.1.2 Peak-Tangent Method

In the *peak-tangent (P-T) method* a common tangent is drawn to two neighboring maxima or minima, and the distance to the intermediate extremum value is measured parallel to the ordinate (t_1, t_2, t_3 in Fig. 2-24). This method can be applied satisfactorily if a linear background is present, but most of the time it is better to check whether a higher order will give more exact results.

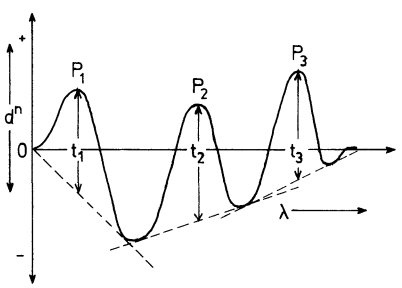

Figure 2-24. Peak-tangent method (PT method), where t_n is proportional to the concentrations of peak P_n.

2.6.1.3 Peak-Zero Method

The *peak-zero (PZ) method* of evaluation is used only in special cases. The vertical distance z from the zero line is measured (Fig. 2-25), which is proportional to the absolute value of the derivative [52]. It is suitable for higher derivatives which have nearly symmetrical signals with respect to the abscissa. This method is also recommended if individual curves overlap in an undistorted state, and one of the signals passes through zero at this λ position (Fig. 2-26).

Distorted main peaks lead to nonlinearity of standard lines or give incorrect results. In this case, it can be helpful to use a *satellite-zero (SZ)* distance for evaluation

(Fig. 2-27 and [61]). On the other hand, if in odd derivatives the positive and negative peak–zero distances are not identical, it is an indication of peak asymmetry, caused by a second superposed signal whose maximum is at a distance from the other maximum, which is smaller than the widest FWHM (Fig. 2-28).

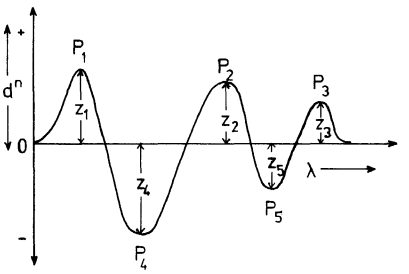

Figure 2-25. Peak–zero method (PZ method), where z_n is proportional to the concentrations of peak P_n.

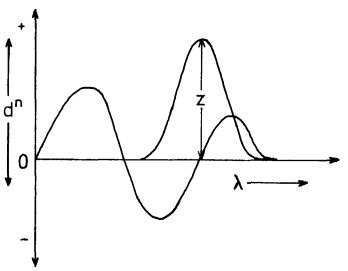

Figure 2-26. Peak–zero evaluation at zero crossing point of another signal.

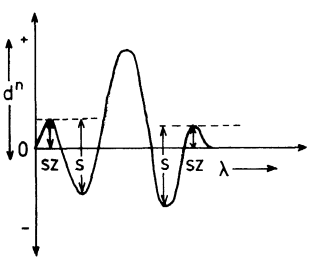

Figure 2-27. Evaluation of satellite peaks. SZ: satellite-zero methods; S: satellite method.

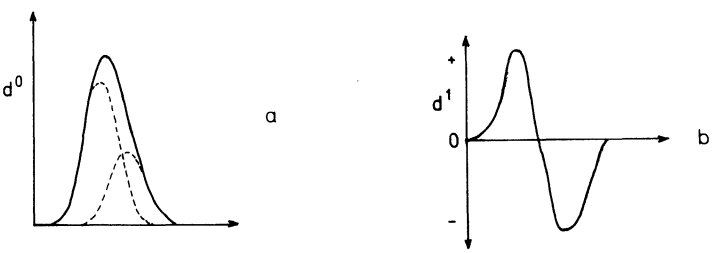

Figure 2-28. Asymmetry caused by two superposed signals.
a) Fundamental spectrum: the distance between the two maxima is smaller than the half width of the sum curve. The slight asymmetry of the fundamental Gaussian signal is negligible. b) First derivative spectrum: The areas below the positive and negative regions of the curve of the first derivative are not equal (indicative of asymmetry in the fundamental curve).

2.6.1.4 Peak-Peak Ratio Method

The *peak-peak ratio (PPR) method* is based on the ratios between pairs of neighboring peaks (p_1/p_2 in Fig. 2-23); if these values differ, then background interference and, therefore, inferior quality of the sample will result. It may also be that P_1 and P_2 in Fig. 2-23 are signals caused by different components of the sample. The ratio of the heights, p_1 and p_2, will be constant as long as the ratio of the concentrations do not vary, even though the absolute concentrations are different and also modified. In other words, the PPR is a characteristic quantity, not only for a pure substance but also for a mixture of components. It enables the analyst to estimate the varying concentrations of a second substance if the initial concentration of the first substance is held constant (as a standard). It is certainly useful when searching for small differences in complicated derivative spectra [33, 62–64]. For further information, see also [61, 65, 66].

2.6.2 Special Methods

2.6.2.1 Half Wave Graphical Illustration

Sometimes only the number or the positions of superposed signals are desired. For this evaluation (the HWG mode), it is practical to use only the positive region of the derivative peaks for a $4n$th order and the negative regions of the peaks for a $4n$-2 order (Fig. 2-29 and [67]).

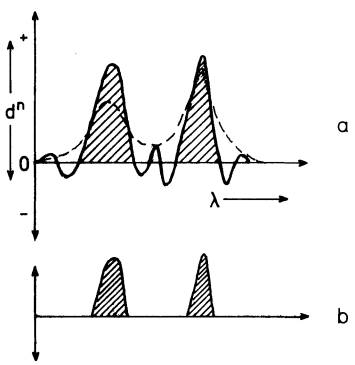

Figure 2-29. Half wave evaluation (HW method).
a) Only positive or only negative regions of the peaks evaluated (without satellites). --- Fundamental signal.
b) Only the region between FWHM and the extremum used (schematic graph).

2.6.2.2 Extended Peak-Peak Ratio Method

In Fig. 2-30, peaks P_1 and P_2 of substances I and II are superposed, but P_3 of substance I is undisturbed. Normally, the overlapping signals cannot be separated; however, it is possible to first estimate the ratio of p_1 and p_3 of pure substance I, and then subtract the amount of p_1 from the overlapping $p_1 + p_2$. The remaining difference gives the quantity required to compute the amount of the second substance [33], and this method is termed the *extended peak-peak ratio (EPPR)* method.

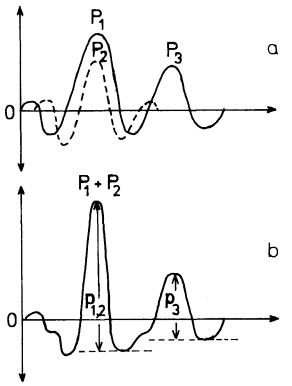

Figure 2-30. Separation of superposed signals by differentiation (EPPR method).
a) Two single derivative signals; b) superposition of signals in a) (see text for explanation).

2.6.2.3 Isosbestic Point Method

When two compounds in a solution are in chemical equilibrium, and both contribute to the absorbance in a particular wavelength region, there will be at least one wavelength where absorbance is a funtion of the total concentration of both species, but does not depend on the relative concentrations. This wavelength is known as the *isosbestic point.*

Derivative spectra sometimes also have isosbestic points. When another signal overlaps these points, an alteration only occurs because of the second substance, and its concentration can easily be estimated.

2.6.2.4 Side-Peak-Side Ratio Method

The PPR method (cf. Section 2.6.1.4) uses the ratio of two neighboring signals. In contrast, the *side-peak-side ratio (SPSR)* method [68] uses only *one* peak, as can be seen in Fig. 2-31. The SPSR method is of advantage if only one wavelength position is to be located. It represents a special variant of the PPR method. The results of computation can be presented either in tabular form [62, 64] or graphicallly, with a very distinct line drawing [68, 69]. Moreover, the data are normalized in a similar manner to the PPR

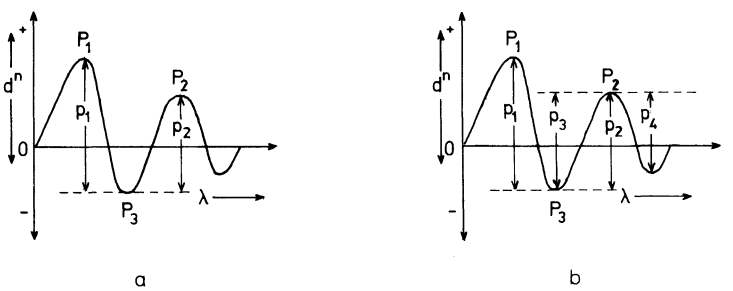

Figure 2-31. Comparison of PPR and SPSR methods.
a) PPR method: P_1 and P_2 need not be adjacent; b) SPSR method: for peak P_2 (maximum) the ratio is $p_3 : p_4$, and for peak P_3 (minimum) the ratio is taken with a negative sign ($p_1 : p_2$).

evaluation, which means that they are independent of the sample concentration (Fig. 2-32).

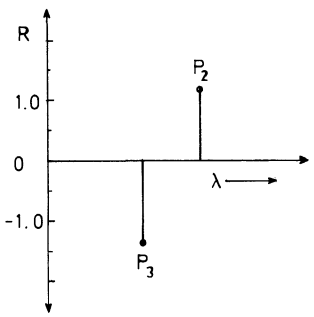

Figure 2-32. Graphical representation of SPSR evaluation of Fig. 2-31 b (peak P_2 and P_3). Both the λ position and the SPS ratio can be easily compared and possible differences noted.

2.6.3 Computational Methods

During the last ten years the development of electronic devices, particularly computers, has progressed dramatically, so that most computational operations and forms of data manipulation are now simpler and can be carried out much more quickly. This evolution also took place in the field of derivative techniques, especially in the evaluation of higher-order derivatives. Computational methods are important for normalizing spectra [70], for comparing complex spectra, and for detecting and locating small differences in derivative signals [71–73].

2.6.3.1 Additive and Subtractive Methods

According to the Lambert-Beer law the absorbance of two different substances is,

$$\text{for substance 1:} \quad A_1 = \varepsilon_1 c_1 l \tag{2-62}$$

and

$$\text{for substance 2:} \quad A_2 = \varepsilon_2 c_2 l \tag{2-63}$$

Analogously, derivatives of the nth order can be written

$$\frac{\mathrm{d}^n (A_1)}{\mathrm{d}(\lambda)^n} = c_1 l \frac{\mathrm{d}^n (\varepsilon_1)}{\mathrm{d}(\lambda)^n} \tag{2-64}$$

and

$$\frac{\mathrm{d}^n (A_2)}{\mathrm{d}(\lambda)^n} = c_2 l \frac{\mathrm{d}^n (\varepsilon_2)}{\mathrm{d}(\lambda)^n} \tag{2-65}$$

Addition and subtraction, respectively, leads to

$$\frac{d^n(A_1 \pm A_2)}{d(\lambda)^n} = l\left[c_1 \frac{d^n(\varepsilon_1)}{d(\lambda)^n} \pm c_2 \frac{d^n(\varepsilon_2)}{d(\lambda)^n}\right] \tag{2-66}$$

If it is assumed that both substances are first mixed and the derivatives are computed afterwards, one comes to identical conclusions. For a 1:1 mixture

$$A = A_1 + A_2 = \varepsilon_1 c_1 l + \varepsilon_2 c_2 l \tag{2-67}$$

and

$$\frac{d^n(A)}{d(\lambda)^n} = \frac{d^n(A_1 + A_2)}{d(\lambda)^n} = l\left[c_1 \frac{d^n(\varepsilon_1)}{d(\lambda)^n} + c_2 \frac{d^n(\varepsilon_2)}{d(\lambda)^n}\right] \tag{2-68}$$

The sum of the derivative spectra of two (or more) individual substances should be equal to the derivatives of the mixture of all components. Upon examination, this statement could, in fact, be confirmed if the substances have a mutual influence and the absorbances are additive. Under these circumstances it is possible, for example, to investigate weak intermolecular forces or the influences of the solvent on the spectra of the components, in that case, the two methods of computation would not give identical results.

Similar to the addition of two (or more) derivatives (*A method*) it is also possible to subtract one or more derivative(s) from a sum of derivatives (*S method*). If

$$A = A_1 + A_2 \tag{2-69}$$

and

$$A_1 = A - A_2 \tag{2-70}$$

then

$$\frac{d^n(A_1)}{d(\lambda)^n} = \frac{d^n(A)}{d(\lambda)^n} - \frac{d^n(A_2)}{d(\lambda)^n} = l\left[c_1 \frac{d^n(\varepsilon)}{d(\lambda)^n} - c_2 \frac{d^n(\varepsilon_2)}{d(\lambda)^n}\right] \tag{2-71}$$

with

$$c = c_1 + c_2 \tag{2-72}$$

and

$$\varepsilon = \varepsilon_1 + \varepsilon_2 \tag{2-73}$$

The S method of evaluation makes it possible, for instance, to separate an unknown derivative spectrum from the sum of this unknown derivative and a well-known derivative.

2.6.3.2 Multiplicative Method

The *multiplicative method* (*M method*) is used for normalization and comparison of derivative spectra or other signals. The highest peaks are usually brought to an identical level and then the signals are subtracted. Aberrations are easily detected, but one must make certain that the differences are not due to residual noise. This can be checked by comparing spectra that have been measured repeatedly, or by multiplying the differentiated base line with the same factor.

2.6.3.3 Partitive Method

As shown in Secs. 2.6.1.4 and 2.6.2.4, the ratio of two single peaks as well as the side-peak-side ratio of one peak are independent of the concentrations of identical substances. This can be shown by measuring out and tabulating the data [33, 62, 64, 68]. Nevertheless , it is easier to divide one spectrum by another, as performed by the *partitive* (*P*) *method.* The ideal result is a straight line. Deviations caused by noise, contaminations or other disturbances are easily detectable. In addition, characteristic distinctions of different substances can be clearly seen.

It can be shown mathematically that the ratio of two spectra is equal to the ratio of the concentrations of the appropriate substances [71, 72]. If

$$P_1 = \frac{\mathrm{d}^n(A_1)}{\mathrm{d}(\lambda)^n} = \frac{\mathrm{d}^n(\varepsilon\, c_1\, l)}{\mathrm{d}(\lambda)^n} = c_1 l \frac{\mathrm{d}^n(\varepsilon)}{\mathrm{d}(\lambda)^n} \tag{2-74}$$

and

$$P_2 = \frac{\mathrm{d}^n(A_2)}{\mathrm{d}(\lambda)^n} = \frac{\mathrm{d}^n(\varepsilon\, c_2\, l)}{\mathrm{d}(\lambda)^n} = c_2 l \frac{\mathrm{d}^n(\varepsilon)}{\mathrm{d}(\lambda)^n} \tag{2-75}$$

then

$$\frac{P_1}{P_2} = \frac{c_1}{c_2} = \text{constant} \tag{2-76}$$

These ratios are independent of the wavelength and are constant over the whole spectral region. The P method is especially suitable for both the control of quality and the detection of small differences in spectra or other curves.

2.6.4 New Variants in the Derivative Method

2.6.4.1 Log *A* Derivatives

A further variant of evaluation, preferred for standardization and comparison, is the *log A method* of derivatization. In the past, logarithms of spectra were used when the absorbance extended to a broader region. Beyond that, spectra of the same substances, but of different concentrations, have an identical shape. The curves are only shifted along the *y*-axis [72]. Theoretically, this advantage, applied to differentiation, leads to congruent signals [72, 73]:

$$\lg(A) = \lg(\varepsilon) + \lg(c) + \lg(l) \tag{2-77}$$

and

$$\frac{d^n}{d(\lambda)^n}(\lg(A)) = \frac{d^n}{d(\lambda)^n}(\lg(\varepsilon) + \lg(c) + \lg(l)) \tag{2-78}$$

In this case, the derivatives are only dependent on the alteration of the molar absorption coefficient ε. The constant concentration c and the thickness of the absorbing layer l are independent of the wavelength (Fig. 2-33). Calculating logarithms of spectra with an absorbance of $0 < A < 1$ serves no useful purpose, because the logarithm to the base 10 of 0 is undefined, and the logarithm to the base 10 of 1 is 0. Therefore such signals should be shifted along the y-axis until the lowest value of A is 1 or higher.

With the log A method, even the smallest differences in the shape of the spectra are obvious. Equations analog to Eq. (2-77) and (2-78) are valid for the natural logarithm ln.

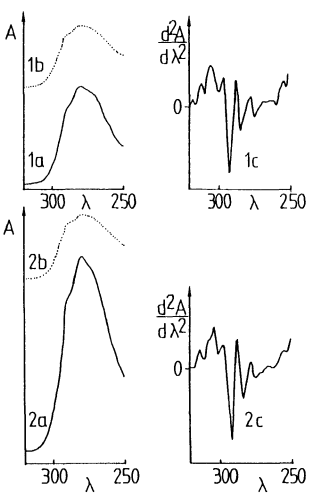

Figure 2-33. log A method.
a) 1a: fundamental spectrum of α-chymotrypsin (concentration: $c = 1$); 1b: logarithm of 1a; 1c: second derivative of 1b; b) 2a: fundamental spectrum of α-chymotrypsin ($c = 2$); 2b: logarithm of 2a; 2c: second derivative of 2b. Although the concentrations of the sample are different, the second derivatives of the log A spectra are practically identical.

2.6.4.2 Differentiation–Integration

In Sec. 2.4 it was shown that differentiation enables one to eliminate unwanted background caused by light scattering or noise. But sometimes the undisturbed fundamental signal is called for. In that case, the differentiated spectrum must be retransformed, step by step, into the fundamental spectrum by integration (D I method).

There are some approximate algorithms for solving determined integrals e.g., the rectangular, trapezoid and tangential formulas, the Simpson and Kepler rules, and integration by polynomials; (see special handbooks of mathematics). In many cases the trapezoid formula will suffice for an integration of derivatives; see Eq. (2-79) and Fig. 2-34.

$$\int_a^b y\,dx \approx \frac{b-a}{2n}\,(y_a + 2y_1 + 2y_2 + \ldots + 2y_{n-1} + y_b) \tag{2-79}$$

Using Eq. (2-79), the error of integration rises with the rising width of intervals i, and is proportional to i^2 if

$$i = \frac{b-a}{n} \tag{2-80}$$

Note that the broader the width of intervals is, the smaller the number of data points will be. It is not a sliding data computation (e.g., the Savitzky–Golay polynomial), but a computation, dependent on the width of intervals. In the sliding data manipulation, the interval width can be varied while the number of data points remains constant. To contrast, broader data intervals correspond to a higher influence on the signal shape (deformation), and a reduced amount of information in the spectra.

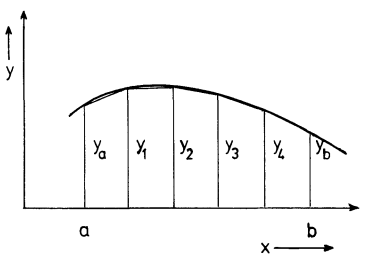

Figure 2-34. Integration by trapezoid formula. *n:* number of equidistant intervals *i* (in this case, $n = 5$). a, b: limits of integration.

2.6.5 Evaluation of Peak Areas

The estimation of the peak area is particularly recommended when the shape of the signals is not symmetrical. One of the simplest way to measure the peak area is to cut out the plotted peak and to weigh the paper in comparison with a well-known area. It is also practicable to plot the curve on a squared graph paper and to count out the number of squares; if the peaks have no horizontal basis, aberration must be taken into consideration (Fig. 2-35).

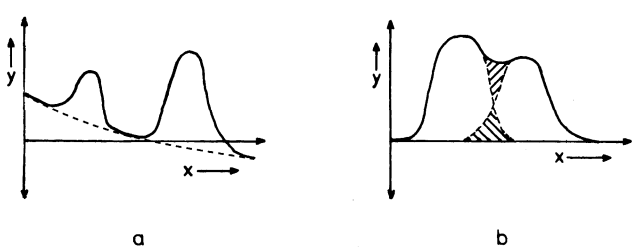

Figure 2-35. a) Baseline correction; b) correction of overlapping – both shaded areas must be equal (schematic drawings).

Nowadays, there are many algorithms to calculate the area below the peaks [74] and also for baseline correction and the correction of overlapping signals. Nevertheless, quantitative results must be reviewed critically because of possible artifacts [75].

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3 Instrumentation

There are numerous methods for generating derivatives of electric signals. Methods are based either on interference in the optical path or interference after optical detection. Methods can be classified as follows: a) Graphical methods; b) Mechanical-electrical methods; c) Optical methods; d) Signal delay methods; e) Analog computing methods; and f) Digital computing methods. The advantages and disadvantages of these differentiation methods will be discussed in this chapter.

3.1 Graphical Methods

The earliest method for the differentiation of spectra or other signals, *the graphical method,* is only suitable for simple curves; furthermore, it requires a great deal of time. The tangents are best drawn by a mirror ruler (Fig. 3-1). The ruler must be positioned in such a way that the mirror-image shows the curve without a break; then the tangent is drawn rectangular to the direction of the ruler.

Another graphical method is applied as follows: To construct the derivative, a pole P is chosen first. The straight lines $\overline{PB_1}$, $\overline{PB_2}$ ... $\overline{PB_n}$ are parallel to the tangents in A_1, A_2 ... A_n. Then A_1', A_2' ... A_n' are the points of intersection of the parallels to the x- and y-axes drawn through B_1 and A_1, B_2 and A_2, and so on (Fig. 3-2).

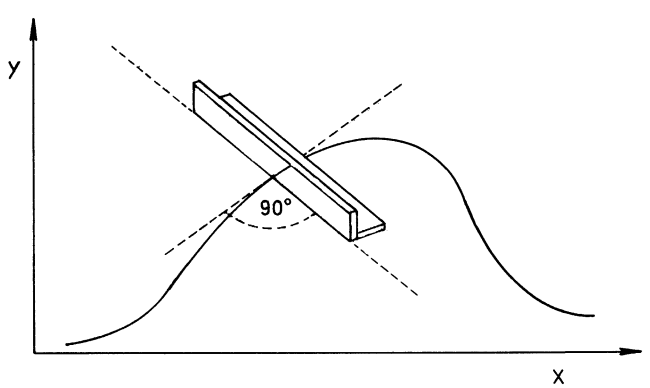

Figure 3-1. Use of mirror ruler for drawing of tangents.

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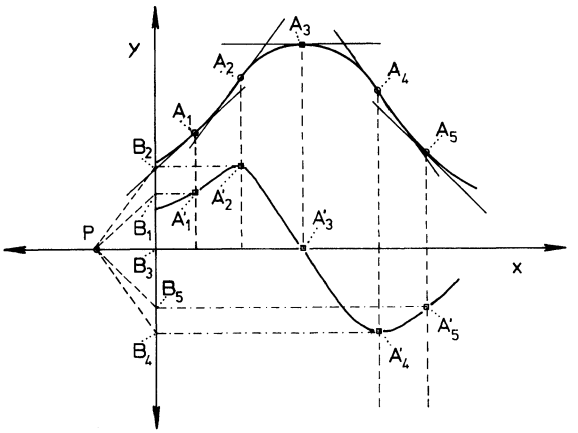

Figure 3-2. Graphical differentiation of a curve to obtain the first derivative.

3.2 Mechanical-Electrical Methods

Olson and Alway [1] described a method to record the first-order derivative. The authors used a recorder with a tachometer generator, coupled with the driving gear of the y-axis, and a lock-in amplifier followed by a filter. For wavelength scanning, the rate of movement of the recorder in the ordinate direction is proportional to the first derivative.

Klein and Dratz [2] produced a 60-Hz signal with a tachometer generator, the amplitude of which was proportional to the ordinate component; amplified with a lock-in amplifier, the signal was rectified, smoothed, and filtered. Only the first derivative could be generated with this method.

3.3 Optical Methods

3.3.1 Modulation Procedures

Modulation methods are based on intervention in the optical path of spectrophotometers. In a sinusoidal wavelength modulation, the main wavelength λ_0 is sinusoidally modulated:

$$\lambda - \lambda_0 = d \sin \omega t \tag{3-1}$$

(λ: auxiliary wavelength, d: vibration range, ω: angular velocity, t: time).

This principle is illustrated in Fig. 3-3. The first derivative is given by the first harmonic component at the same frequency applied for modulation. The resulting signal

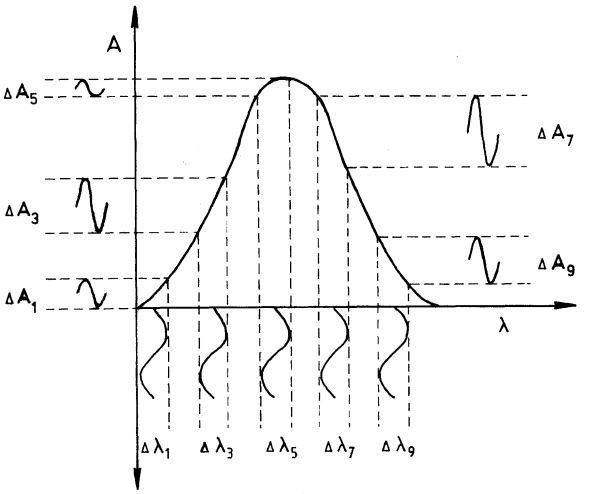

Figure 3-3. Sinusoidal wavelength modulation of an analytical peak (schematic drawing). $\Delta\lambda_1 \ldots \Delta\lambda_9$ sinusoidal modulation; $\Delta\lambda_1 \ldots \Delta\lambda_9$ resultant modulation in intensity.

is then proportional to the difference in intensity of absorptions between the modulation interval $\Delta\lambda$. In other words, the resulting signal is proportional to the slope of the curve between λ and $\lambda + \Delta\lambda$.

Often a spectral band is superposed on a broad and unstructured background, which, by itself, will generate a "ripple" (alternating current component) in the photocurrent at the same frequency as that of the applied wavelength modulation interval (Fig. 3-4). If, at twice the applied modulation frequency, a spectral line is also present

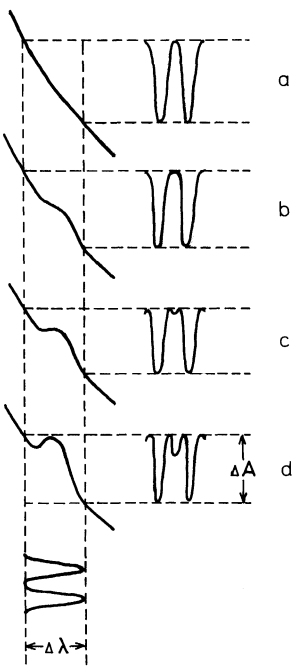

Figure 3-4. Wavelength modulation for detection of a weak spectral signal superposed on an intense background (after [3]. a) pure background, b–d different signals on background. The left vertical series are the fundamental spectra, the right series the resulting $\Delta\lambda$ modulated spectra).

in the modulation interval, a second harmonic component will be generated, which is directly proportional to the second derivative.

A pure sine curve is only produced at the outlet of the multiplier, or another detector, if the background is linear over interval $\Delta\lambda$. For real backgrounds it will not be strictly true, but in most cases this is a good approximation because the modulation interval is usually much smaller than 1 nm.

The next step is to separate the second harmonic component from the often much larger first harmonic component. This is done with a phase-sensitive AC amplifier (lock-in amplifier) which is synchronized with the scan and tuned to the second harmonic frequency (i.e., twice the modulation frequency).

Let us consider the potential for wavelength modulation. In 1953 Hamond and Price [4] proposed the wavelength modulation principle based on a vibrating Littrow mirror. One year later the photobiologists French, Church and Eppley [5] adapted it to study first-order derivative spectra of photosynthetic systems in the visible region [6–10]. In the years that followed, various modulation techniques were employed to generate derivatives of different spectra; a review is given in Table 3-1.

In addition to sinusoidal modulation, square wave modulation [3, 33] was also sometimes used. Generally, the applied modulation frequency lies between 50 and

Table 3-1. Chronological overview of references on modulation techniques for differentiation of spectra.

Modulation Technique	Author	Year	Reference
Vibrating mirror	Bonfiglioli and	1963	[11]
	Brovetto	1964	[12]
	Snelleman	1968	[13]
Oscillating mirror	Aramu and Rucci	1966	[14]
	Bonfiglioli et al.	1967	[15]
	Overend et al.	1967	[16]
	Evans and Thomson	1969	[17]
Rotating mirror	Stauffer and Sakai	1968	[18]
Vibrating quartz plate	Snelleman et al.	1970	[19]
Mechanical oscillating quartz plate	Sneddon et al.	1982	[20]
Quartz refractor plate	Gilgore et al.	1967	[21]
Beam deflecting plate	McWilliam	1959	[22]
	Perregaux and Ascarelli	1968	[23]
	McWilliam	1969	[24]
	Elser and Winefordner	1972	[25]
	Fowler et al.	1974	[26]
Vibrating slit	Balslev	1966	[27]
Oscillating slit	Williams and Hager	1970	[28]
Laterally oscillating monochrome entrance slit	Hager	1973	[29]
	Stäudner	1976	[30]
Oscillation of monochromator at low frequency (prism or grating)	O'Haver et al.	1973	[31]
	Green and O'Haver	1974	[32]

1000 Hz; in special cases the oscillation frequency of prisms or gratings can be lower. The modulation intervals are usually small, i.e., not more than 0.01 nm.

Other modulation techniques are oscillation (tilting) of an interference filter [3] and modulation of the electron beam scan pattern in a vidicon or image-disk-sector photomultiplier spectrophotometer [34]. This was the first nonmechanical wavelength modulation. Wavelength modulation induces a synchronous modulation of the amplitude. If these intensities are expanded, for instance, in the form of a Taylor series in λ_0, and the powers of the sine functions are expressed as sine and cosine functions of the corresponding multiple angles, then the derivatives can be obtained from the Fourier coefficients (see Sec. 2.1.3.3) of these series. The second derivative is obtained from the second harmonic of the induced intensity.

Theoretically, the nth harmonic results in the nth derivative, but only derivatives of the first or second order have actually been generated by this modulation technique. This is a considerable disadvantage if the overlapping signals to be resolved requires higher-order derivatives ($n > 2$). Also, intervention in the optical path along with lock-in amplification is relatively complex. On the other hand, a low noise level is inherent to the derivative spectra obtained through the modulation technique. Another essential point is that the amplitude of the second harmonic component will remain practically unchanged, even if the background fluctuates or drifts in intensity.

Further theoretical considerations to sinusoidal wavelength modulation are given by Aramu and Rucci [14], Bonfiglioli and Brovetto [12], Fell [35], Hager and Anderson [29, 36], O'Haver [3], and Williams and Hager [28].

3.3.2 Dual-Wavelength Spectrophotometry

The method of *dual-wavelength spectrometry* was already proposed by Chance in 1951 [37]; Giese and French [6] constructed a double-beam dual-wavelength spectrophotometer for this purpose. But the merits of developing it were not pointed out until 1969 and later by Shibata et al. [38–41].

The principle is relatively simple. The beam of light entering the system is divided equally between two monochromators that are driven simultaneously , but which produce monochromatized radiation that differs in wavelength by a constant amount $\Delta\lambda$. By means of a chopper the two beams pass alternately through a single sample cell; the intensity of the transmitted radiation is then measured by multipliers. After amplification, the signals are directed to a subtraction module whose output is connected to the y input of a recorder; the x-axis of the recorder is run synchronously with the wavelength scan. If $\Delta\lambda$ between both monochromators is sufficiently small, approximately 1–2 nm, the first derivative, with respect to the wavelenght, is obtained. The adjusted λ interval of the two monochromators affects the resolution of the electric signals. The smaller $\Delta\lambda$, the better the resolution. Unfortunately, only the first derivative can be generated directly by this low-noise optical technique; higher-order derivatives must be computed in other ways.

3.4 Subtraction of Spatially or Temporally Delayed Spectra

3.4.1 Spatial Delay

The spatial shift of identical spectra, followed by subtraction of the signals, can occur in different manners. The principle of this method can be seen in Fig. 3-5.

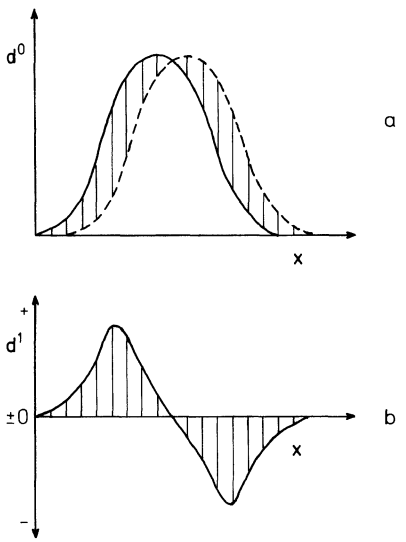

Figure 3-5. Subtraction of spacially delayed signals. a) Fundamental spectrum; b) first derivative.

3.4.1.1 Delayed Recording

In the simplest way possible, the spectrum is first scanned and recorded. After shifting the pen of the recorder by $\Delta\lambda$, the spectrum is again scanned and the differences of both curves are measured manually as a function of λ. The result of this manipulation leads directly to the first-order derivative. The same results are obtained with only a single scan if it is recorded by a two-channel recorder with two pens at distance $\Delta\lambda$ [42].

3.4.1.2 "Intelligent Recorder"

More elegant results can be obtained by using an "intelligent recorder", that is, a recorder with an integrated microcomputer. After a scanning of the spectrum and analog-to-digital conversion, the plotted signal can be shifted point by point, and the delayed spectra subtracted. Once the first derivative of the basic spectrum has been obtained, each step can be repeated, thereby also making it possible to generate higher-order derivatives.

3.4.1.3 Dual-Wavelength Delay

The delay of spectra can also be achieved by two monochromators, as already pointed out in Sec. 3.3.2.

3.4.1.4 Cassette-Tape Delay

Inoue et al. [43] has developed a derivative technique using a modified cassette recorder connected to a spectrophotometer. The same absorption spectrum was recorded on two separate parallel channels of a magnetic-tape cassette as frequency modulated (FM) analog signals with a slight shift in wavelength (~1 nm). Then the difference between the output signals from these channels was recorded (Fig. 3-6).

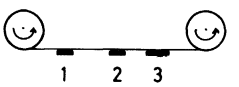

Figure 3-6. Schematic drawing of a modified tape recorder, where 1 is the first recording head, 2 is the second recording head (the distance to 1 is variable), and 3 is the playback double head.

Theoretically, not only the first derivative but also higher-order derivatives can be generated by connecting two or more of these apparatus in series. But this has not been attempted to date, perhaps because of the difficulties arising with synchronization and interference due to signal noise.

3.4.2 Temporal Delay

If the scan speed of the monochromator is constant – which is the case if a grating instead of a prism is used for the dispersion of light – then the wavelength position is linearly proportional to time. In that case, derivatives are obtained by *temporally delayed signals* by dividing the output of the photomultiplier into two equal parts. One signal passes directly to the subtraction unit, and the other signal is initially delayed in time by an electronic circuit [44].

3.5 Analog Differentiation

Analog (eletronic) and digital (numeric) differentiation are nearly the only methods that are being used currently, especially when higher-order derivatives are needed. Therefore, these two techniques will be treated here in detail.

3.5.1 The First Experiments with Electronic Differentiators

In 1953, Collier and Singleton introduced electronic (analog) differentiation [45]. They developed an electronic device with wireless valves and patented their invention [46]. The

apparatus was suited for generating first- and second-order derivatives. Some years later, in 1959, Collier and Panting connected a logarithmic module in series [47]. This supplementary unit enabled then to estimate, for example, 0.5 CH_3 groups per 100 CH_2 groups in polyethylenes by IR derivative spectroscopy.

In the same year, Martin [48] had given the theoretical basis for the assumption that higher-order derivatives ($n > 2$) have the potential for higher resolution compared to lower-order derivatives, but he himself used only a simple (passive) RC module (d^2) with connected valve amplification (Fig. 3-7).

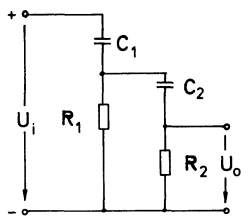

Figure 3-7. Simple (passive) second-order derivative modules, where C_1 and C_2 are capacitors, and R_1 and R_2 resistors. Resistance: $R_1 = 2$ KΩ; $R_2 = 500$ Ω; capacitance $C_1 = 100$ μF; $C_2 = 100$ μF.

Amplifying the module's output is advisable because the signal voltage drops with differentiation. Moreover, the use of the valve has the added advantage that successive differentiating circuits are effectively isolated from one another.

In contrast to the techniques discussed so far, analog differentiation simplified the differentiation of curves or other electric signals; no intervention in the optical path of the spectrophotometer was necessary and the device could also be used for every other apparatus that converts a mechanical, optical, or other quantity into an electric signal.

The invention of the *transistor* by Bardeen, Brattain, and Shockley in 1947, was of the utmost importance for electronic circuits. This active electronic structural member requires no heating current, as is the case for electronic tubes, and it is much smaller and also much cheaper. In the 1960s, the "old" electronic valve was also replaced in derivative devices (see Fig. 3-8). This circuit is not yet perfect, because the differentiator and the amplifier are not independent of each other. Better results can be obtained if the amplifier has a high-resistance input, as realized by a *field-effect* transistor (see Fig. 3-9).

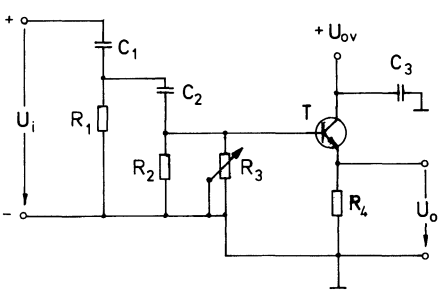

Figure 3-8. A second-order (passive) derivative module with emitter-transistor amplifier. C_1, C_2, C_3: capacitors; R_1, R_2, R_4: resistors; R_3: variable resistor; $+U_o$: operating voltage of the amplifier; T: npn transistor; U_i: input voltage of the module; U_o: output voltage of the module.

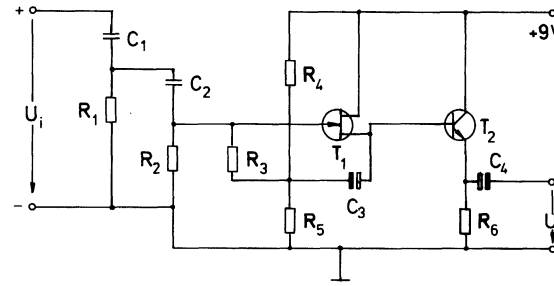

Figure 3-9. A second-order derivative module with impedance converter and emitter-transistor amplifier. C_1, C_2: ceramic capacitors; C_3, C_4: low-voltage electrolyte capacitors (tantalic); $R_1 \ldots R_6$: carbon thin-film resistors; T_1: field effect transistor (e.g., BF 245 (TI)); T_2: npn-Si-HF transistor (e.g., BF 115); U_i: input voltage; U_o: output voltage.

3.5.2 Operational Amplifiers

The application of transistors in combination with resistors and capacitors in *integrated circuits* (*IC's*), gave rise to even smaller and more efficient derivative modules in the 1970s (to be regarded as a "black box"). In an area of 1-2 mm^2, this modern integrated active element takes over the functions of eight to ten transistors as well as of several resistors and capacitors. To construct an operational amplifier, only two supplementary resistors are necessary in the simplest case (Fig. 3-10).

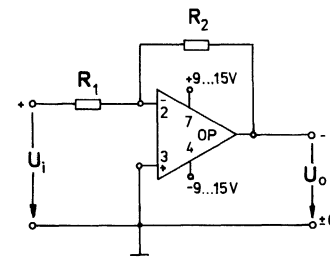

Figure 3-10. Inverting amplifier. Pin 2 (marked with a minus sign) is the inverting input of the operational amplifier (OP), pin 3 (marked with a plus sign) is the noninverting input. The OP is supplied with an operating voltage, connected to pin 4 and 7.

The ratio of the resistances determines the ratio of amplification V_a:

$$V_a = \frac{R_2}{R_1} \tag{3-2}$$

and

$$U_o = -U_i \frac{R_2}{R_1} \tag{3-3}$$

The circuit in Fig. 3-10 is known as an *inverting module;* it inverts a positive voltage to a negative voltage and vice versa.

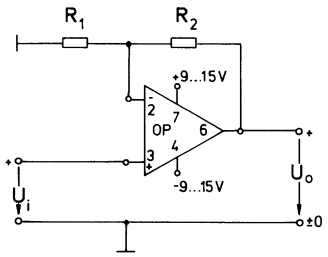

Figure 3-11. Noninverting amplifier.

Another possibility is to use the IC as a *non-inverting amplifier.* Then the input and the output have the same sign and the resistors R_1 and R_2 must be connected differently (Fig. 3-11). The ratio of amplification is now

$$V_a = \frac{R_2 + R_1}{R_1} = 1 + \frac{R_2}{R_1} \tag{3-4}$$

and

$$U_o = U_i \frac{R_2 + R_1}{R_1} \tag{3-5}$$

and the input resistance is essentially higher than in the case of the circuit in Fig. 3-10 (inverting amplifier).

If R_1 and R_2 in Fig. 3-10 are equal, V_a is practically 1 (no-load working); this means that there is no amplification, but that the module has a relatively high-input resistance and a low-output resistance. Such a circuit, which separates two modules electrically (*galvanic separation*), is called an *impedance converter.* The procedure is very important for quality of spectra, especially when generating higher-order derivatives; we shall deal with this problem later.

R_1, and R_2, and grounding can often be obmitted, and output pin 6 in Fig. 3-11 can be directly connected to pin 2 (direct feedback connection). This will also result in a high-resistance, noninverting impedance converter (Fig. 3-12), but in a simpler one. Impedance converters using ICs which contain field-effect transistors have input resistances of up to 10^{12} Ω. This means that the electrical separation effect of these modules is extremely high.

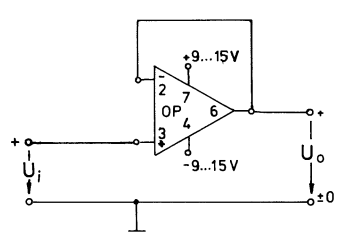

Figure 3-12. Impedance converter using IC 741; the DC voltage supply is connected to pin 4 and 7.

3.5.3 Differentiators and Integrators

The simplest passive differentiator consists of only two parts: a capacitor, C, and a resistor, R, as can be seen in Fig. 3-13. This four-terminal high pass can be combined with an IC, replacing resistor R_1 of the amplifier in Fig. 3-10 with a capacitor C_1 (Fig. 3-14).

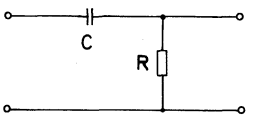

Figure 3-13. Simplest passive differentiator.

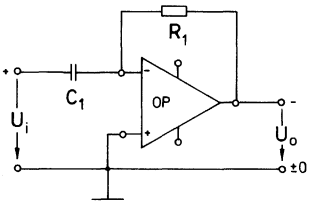

Figure 3-14. Basic circuit of an inverting active differentiator.

The transmission function for this circuit is

$$U_o = -RC\frac{du_i}{dt} \qquad u_i \text{ complex input potential (AC and DC)} \tag{3-6}$$

The result is a simple and modern inverting differentiator. If C_1 and R_2 are changed, the electric behavior will be the same as the inverse function of the differential, namely the integral (Fig. 3-15). The same operation can be applied to noninverting modules. In the circuit of the noninverting amplifier (Fig. 3-11) resistor R_1 is replaced by capacitor C_1 (Fig. 3-16).

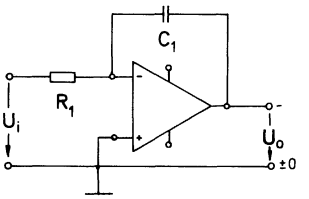

Figure 3-15. Simple inverting active integrator.

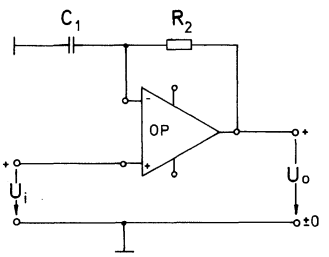

Figure 3-16. Simple noninverting active differentiator.

An important quantity, for differentiators as well as for integrators, is the *time constant* τ:

$$\tau = R_1 C_1 \text{ [s]} \tag{3-7}$$

It determines the quality of the derivatives with respect to resolution of the signal and background noise. A small value for τ results in a high resolution of the irregularities in the curve, but also in a high level of noise. A high value for τ has the opposite effect. Since both high resolution and low noise are worth striving for, something must be done to minimize the noise. The circuits given in Fig. 3-14 and 3-16 are certainly suited to generate the first derivative and perhaps also the second, but they are not suitable for higher-order derivatives. With higher-order derivatives, the noise of the output signal rises to such a degree that the effective signal is almost lost.

One possibility for lowering the level of disturbing noise in analog data is to use electronic analog filters that are both integrated in the differentiating circuit and connected in series to the computing module.

3.5.4 Analog Filters

Electronic filters have to exclude unwanted frequencies. This is simply a frequency-dependent resistor which is a combination of resistors and capacitors, inductors and capacitors, or resistors and inductors.

3.5.4.1 Passive Filters

Some basic types of electronic devices and their frequency characteristics can be found in Figs. 3-17 to 3-23.

Low-pass filters are permeable to low frequencies ($f < f_c$) and impermeable to high frequencies ($f > f_c$) (Fig. 3-17). The cut-off frequency, f_c, is defined as the frequency at which the amplitude of the input signal is lowered to 70.7%.

$$\frac{U_2}{U_1} = \frac{R}{\sqrt{2R^2}} = \frac{1}{\sqrt{2}} = 0.707 \tag{3-8}$$

The cut-off frequency can be computed by

$$f_c = \frac{1}{2\pi RC} \tag{3-9}$$

If the time constant τ (see Eq. 3-7) is much higher than the time period (T) of the signal

$$\tau \gg T \tag{3-10}$$

then the circuit has the property of an integrator

$$U_2 = \frac{1}{RC} \int_0^t u_1 \, dt = \frac{1}{\tau} \int_0^t u_1 \, dt \tag{3-11}$$

The dimensions of the quantities are as follows: τ: seconds (s); C: farads (F); R: ohms (Ω); U: volts DC (V); u: complex potential (DC and AC) (V). On the other hand, the signals are blocked by *high-pass filters* if $f < f_c$, but pass the devices if $f > f_c$ (Fig. 3-18).

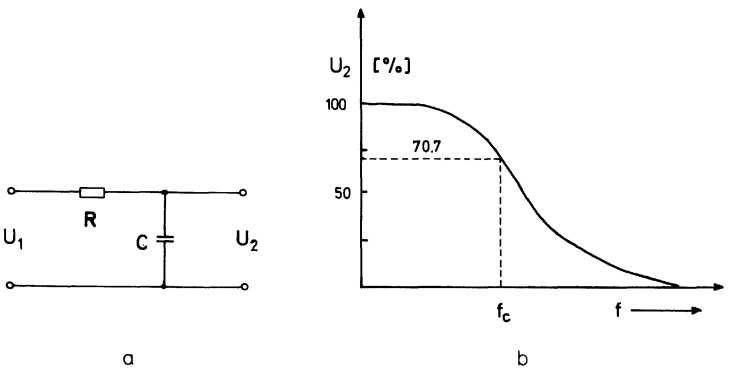

Figure 3-17. Passive low-pass filter.
a) Circuit; b) frequency response curve. U_1: input voltage; U_2: output voltage; f: frequency; f_c: cut-off frequency (f-axis on a logarithmic scale).

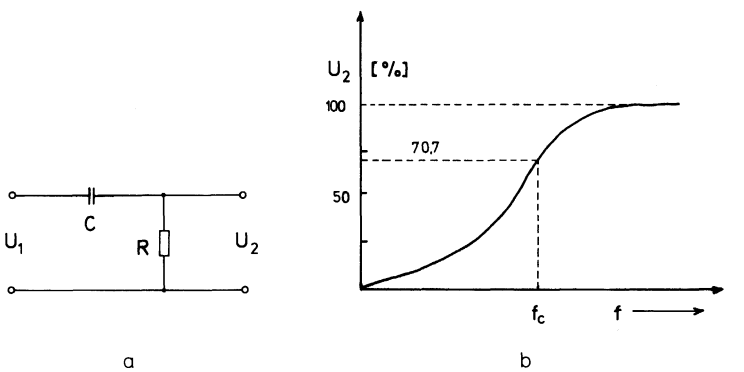

Figure 3-18. Passive high-pass filter.
a) Circuit; b) frequency response curve (f-axis on a logarithmic scale).

When

$$\tau \ll T \tag{3-12}$$

then

$$U_2 = RC\frac{du_1}{dt} = \tau\frac{du_1}{dt} \tag{3-13}$$

The circuit has the property of a differentiator.

The *band-pass filter* is composed of a low-pass and a high-pass filter (Fig. 3-19a). The band width, b, is the frequency band that passes the filter nearly undisturbed between the low frequency, f_l, and the high frequency, f_h; f_m is the middle frequency (Fig. 3-19b).

The *band-rejection filter* has the reverse function of a band-pass filter and consists of a high-pass filter, followed by a low-pass filter (Fig. 3-20).

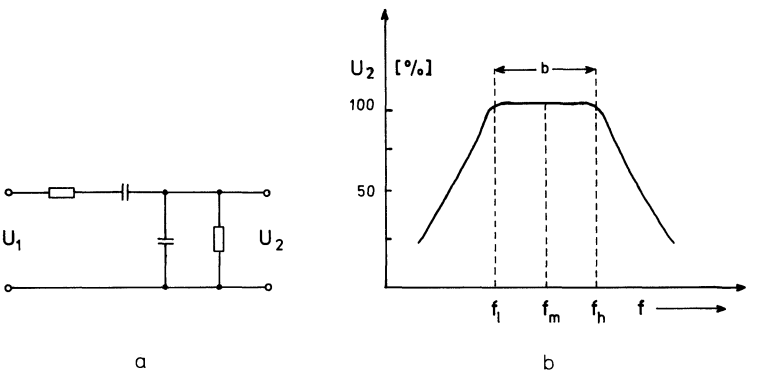

Figure 3-19. Passive RC band-pass filter.
a) Circuit; b) frequency response curve. f_l: low cut-off frequency; f_m: middle cut-off frequency; f_h: high cut-off frequency; b: bandwidth (f-axis on a logarithmic scale).

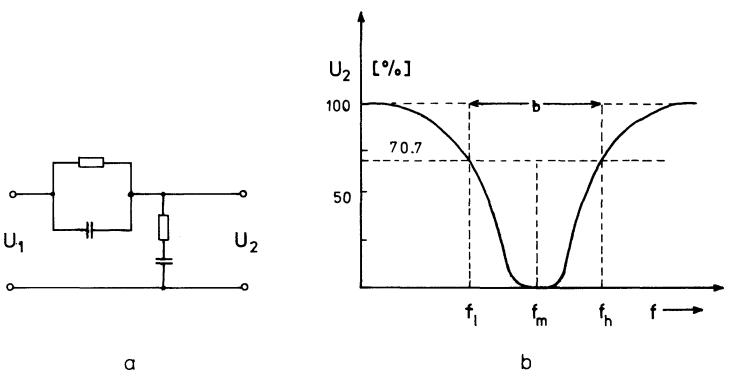

Figure 3-20. Passive CR band cut-off.
a) Circuit; b) frequency response curve. f_l: low cut-off frequency; f_m: middle cut-off frequency; f_h: high cut-off frequency; b: bandwidth (f-axis on a logarithmic scale).

All the filters described above are *passive filters,* which means that they have no amplifying modules. Only resistors and capacitors were used. Other inductive circuits are similar but are not commonly used, because, as in the case of low-pass filters, in the region of 10 Hz and lower, the inductances must be high and therefore the required coils would need to have relatively large dimensions. Some examples are given in Figs. 3-21, 3-22, 3-23.

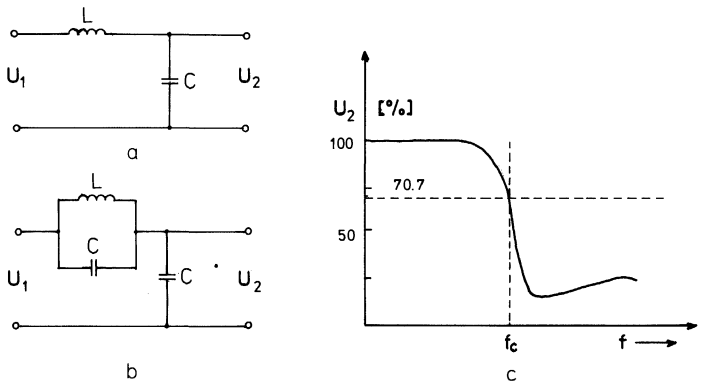

Figure 3-21. Passive LC circuits.
a) Passive LC low-pass filter; b) passive LC low-pass double sieve; c) frequency response curve of (b) (*f*-axis on a logarithmic scale).

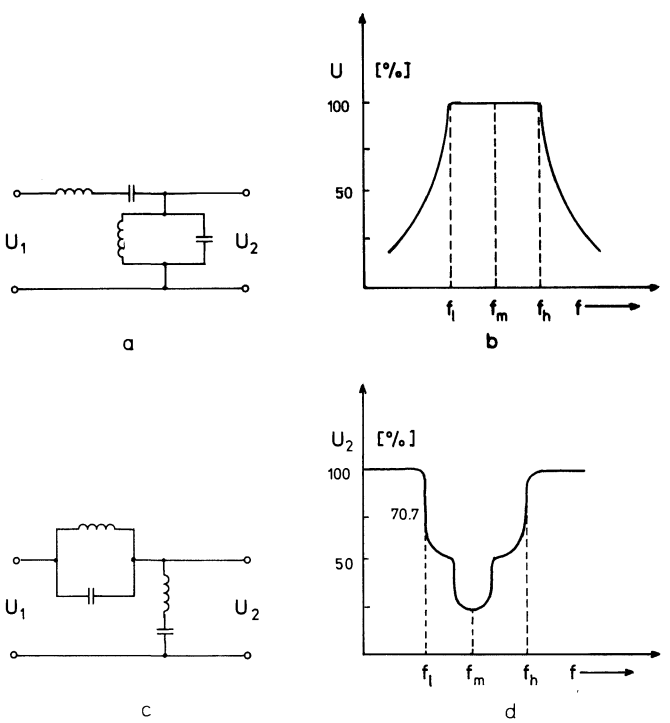

Figure 3-22. Passive LC circuits.
a) LC band pass;
b) frequency response curve of (a); c) LC band cut-off; frequency response curve of (c) (*f*-axis on a logarithmic scale).

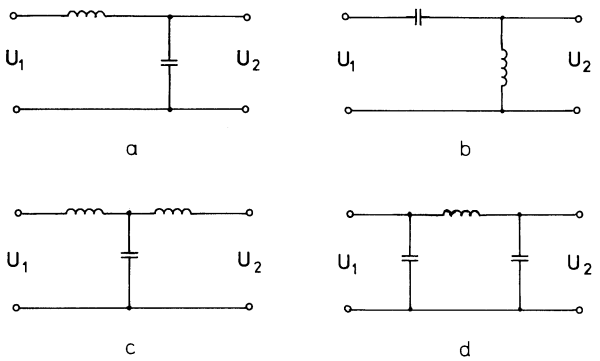

Figure 3-23. Passive LC and CL circuits.
a) Simple LC low-pass filter,
b) CL high-pass filter, c) LC-T low-pass filter, d) LC-π low-pass filter.

The order of a filter indicates how many filters are coupled in a series (Fig. 3-24). The higher the order of the filter, the higher the steepness of the cut-off. The sieving effect is highest when all capacitors and resistors are all of the same value.

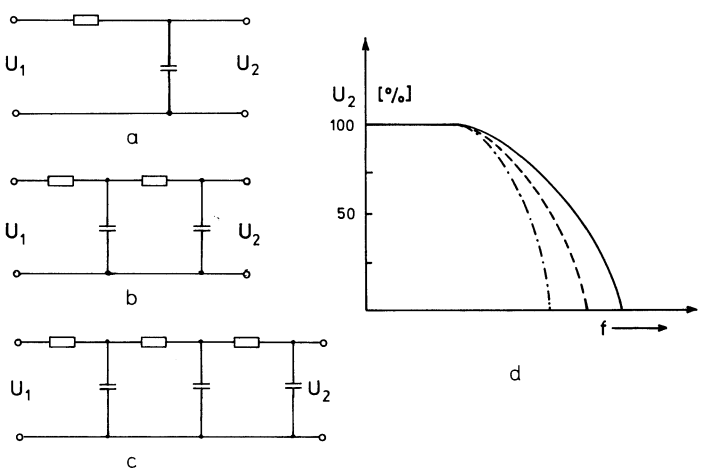

Figure 3-24. Passive higher-order low-pass filters.
a) First-order low-pass filter; b) second-order low-pass filter; c) third-order low-pass filter; d) frequency response curve of (a) ——, (b) – – –, and (c) – · – · – · (*f*-axis on a logarithmic scale).

3.5.4.2 Active Filters

In the previous Section 3.5.4.1 we learned that capacitors and inductors (coils) are frequency-dependent elements. For low frequencies, it is easier to pass inductors, and for high frequencies, to pass capacitors. RC filtershave the disadvantage that for DC sieving a drop in potential occurs. Therefore it is suitable to combine passive filters with amplifiers. Nowadays, this idea can be easily realized with IC's (Fig. 3-25).

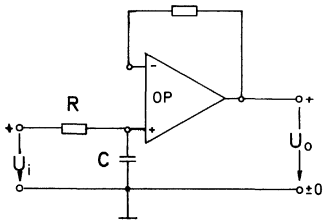

Figure 3-25. Simple (active) first-order low-pass filter, noninverting. $\tau = RC$ [s]; $f_c = 1/2\,\pi RC = 1/2\,\pi\tau$. For example, if $R = 10^4\ \Omega$, $C = 10^{-6}$ F, then $f_c \ldots 15.9$ [Hz].

Real spectra produced by spectrophotometers or other signal sources consist of the effective signal and the overlapping noise, the frequency of which is generally higher than that of the spectra. Therefore, to eliminate disturbing frequencies, low-pass (or sometimes band-pass) filters are needed. Thus only these kinds of filters will be treated in the following. A low-pass filter with ideal frequency characteristics must allow all frequencies to pass without attenuation (up to the cut-off frequency) and must then abruptly block off all higher frequencies (Fig. 3-26).

This ideal filter can only be approximated. The frequency response is determined by the dimensions of the resistors and conductivities. Generally, a filter with a linear transmission band has a flat transition band, whereas a filter with a relatively sharp transition band has a wavelike transmission band (Fig. 3-26b)

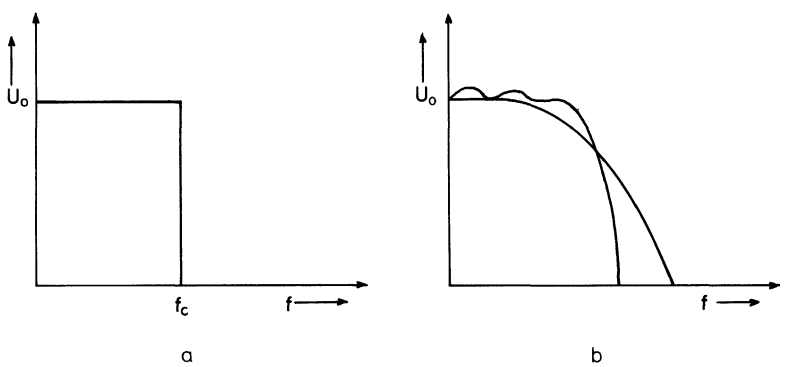

Figure 3-26. Frequency response curve of a low-pass filter.
a) Ideal; b) actual.

The advantages and disadvantages of four low-pass filters are listed below (Fig. 3-27):

a) Gauss function
Advantage: An impulse causes no overoscillation.
Disadvantage: In the permeable band there is a strong drop in amplitude and the transition band is flat. It is "passive" because there is no amplification.

b) Bessel function
Advantage: Attenuation is small and the flattest group run is observed, which means that signals with different frequencies have the smallest differences in time attenuation. This is important because overlapping signals are not distorted.
Disadvantage: Minor overoscillation; the transition band is flat.

c) Butterworth function
Advantage: Its signal is very flat in the permeable band.
Disadvantage: An impulse causes overoscillation.

d) Tschebyscheff function
Advantage: The transition band is very steep.
Disadvantage: The transmission has a wavelike shape.

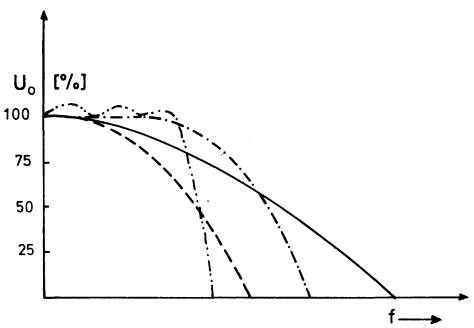

Figure 3-27. Frequency response curves of four low-pass filters: (----) Gauss; (——) Bessel; (- · - · -) Butterworth; (- · · - · · -), Tschebyscheff.

To smooth spectroscopic signals, mainly the Bessel or Butterworth type of filter is used. The steepness of the transition band can be increased by two or more filters connected in series (order of filter). Higher-order filters ($n > 2$) are combinations of first- and second-order modules. The combination of passive filters with amplifiers can either be arranged in a series or integrated in the feedback circuit of the amplifier; a combination of both methods is also possible (Fig. 3-28). Figure 3-29 shows a noninverting low-pass filter. In this case a high-pass filter in the feedback has the function of a low-pass filter and vice versa. In filters with multiple feedback, amplifiers operate in the inverting mode (Fig. 3-30).

A second-order, noninverting low-pass filter (Figs. 3-31, 3-32) can be obtained through a small alteration of the circuit in Fig. 3-25.

It is advisable to connect in series a module which has a DC way to the ground (Fig. 3-33). Then the bias (grid potential), which is necessary for the "real" low pass, will be guaranteed. In this type of filter the DC signals, as well as the alternating current (AC) signals pass the module. When the DC contribution to U_i is to be blocked, a capacitor should be added to the circuit (Fig. 3-34). In that case DC grounding is mandatory.

There are many different low-pass filters but in this chapter we describe only those modules that are necessary for the understanding and application of higher-order differentiation. To construct a home-made device, special handbooks must be consulted (e.g., [49–51]).

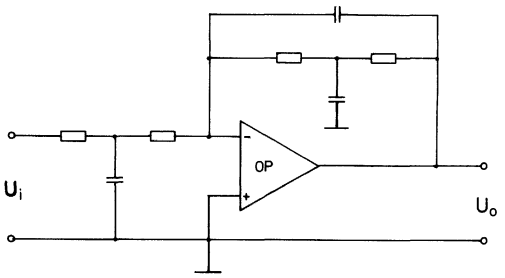

Figure 3-28. First-order low-pass filter with low-pass network in series and in the feedback (inverting).

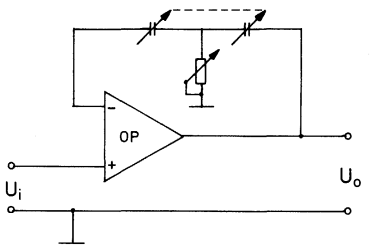

Figure 3-29. First-order active low-pass filter with high-pass filter in the feedback (noninverting).

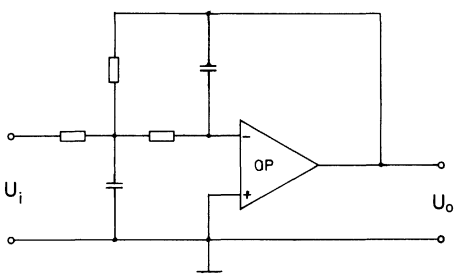

Figure 3-30. Active low-pass filter with double feedback system (inverting).

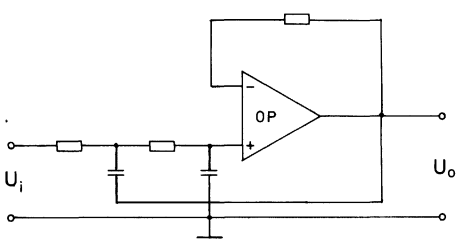

Figure 3-31. Sallen-Key second-order low-pass filter (noninverting; amplification 1).

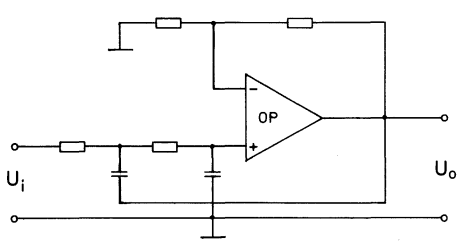

Figure 3-32. Sallen-Key second-order low-pass filter (noninverting; amplification >1).

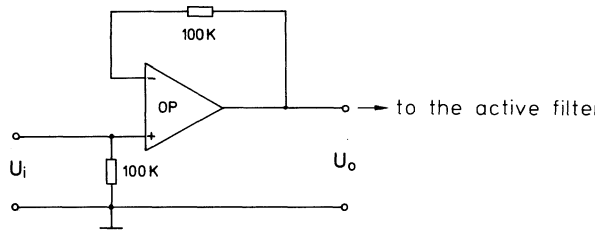

Figure 3-33. DC grounding.

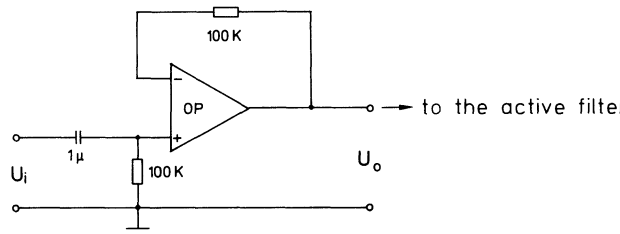

Figure 3-34. The DC part in U_i is blocked by the 1 µF capacitor; grounding over the 100 KΩ resistor is open for DC.

3.5.5 Differentiators with Integrated Filters

The next step is to obtain *practical* differentiators, which involves combining differentiators with filter modules. The latter can be arranged in series or even integrated in the differentiator itself.

For most practical purposes, these differentiators have too much high-frequency noise in their output – after all, a differentiator is nothing more than a high-pass filter – and therefore the circuit must be modified. One improvement is the addition of a resistor in front of the capacitor (cf., Fig. 3-14 and Fig. 3-35). The upper cut-off frequency of this differentiator is

$$f_{c_h} = \frac{1}{2\pi R_1 C_1} \tag{3-14}$$

and the low cut-off frequency, as analogous expression, is

$$f_{c_l} = \frac{1}{2\pi R_2 C_1} \tag{3-15}$$

A further step in this direction is to place a capacitor parallel to the resistor R_2 (Fig. 3-36). If this differentiator is combined with a low-pass filter and an impedance converter, we have an effective network (Fig. 3-37) with which higher-order derivative devices can also be constructed.

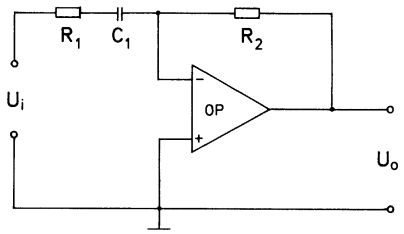

Figure 3-35. Differentiation with added resistor R_1 in series for smoothing of noise (inverting).

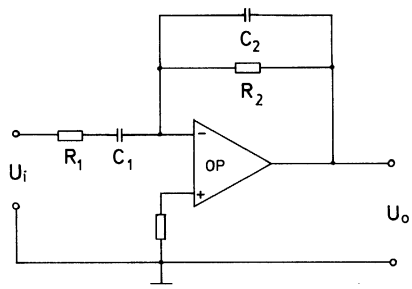

Figure 3-36. Improved inverting differentiator; capacitor C_2 is added to the feedback system to lower the noise level.

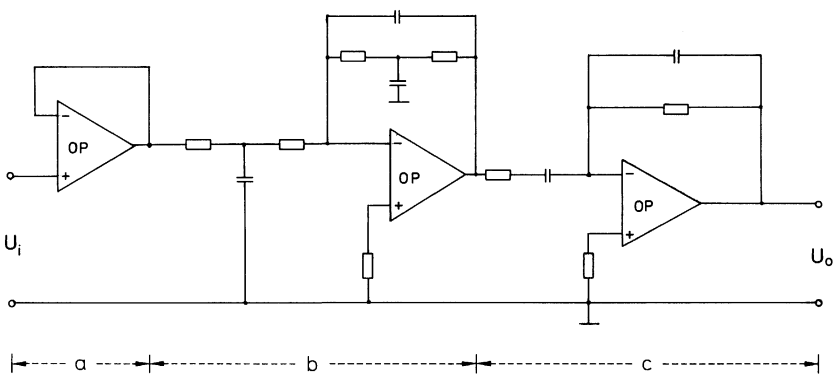

Figure 3-37. Differentiator (c) connected in series with an impedance converter (a) and an active low-pass filter (b).

3.5.6 More Complicated Specialized Differentiators

Two especially interesting forms of differentiators will now be described. The first (Fig. 3-38) combines the signal and its first derivative. In this case the simple transmission function in Eq. (3-6) changes to

$$U_o = -\frac{U_i R_0}{R_2} - R_0 C \frac{du_i}{dt} \tag{3-16}$$

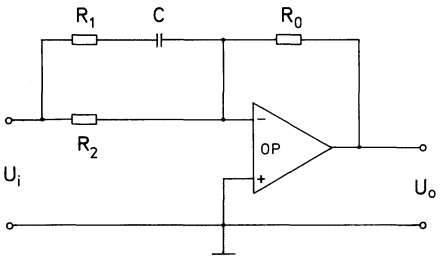

Figure 3-38. Circuit for adding signal voltage to its first derivative [52].

The second arrives at differentiation by subtracting the input signal from its integral. In this elaborate circuit the input noise of amplifier A_1 is integrated, which means that it is filtered by a low-pass filter, and the input noise of amplifier A_2 is added (Fig. 3-39).

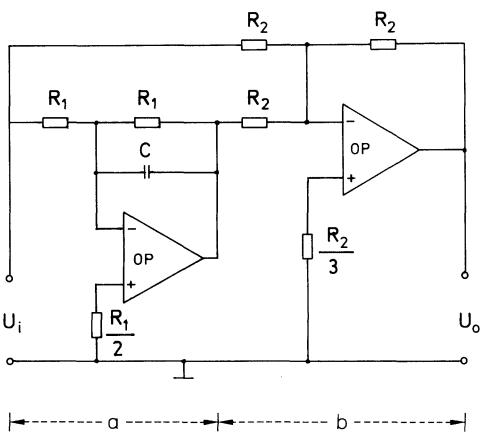

Figure 3-39. Differentiation by subtracting the input signal from its integral. a) integrator; b) subtractor (according to [52]).

The transmission function for this circuit reads

$$U_o = -R_1 C \frac{du_i}{dt} \tag{3-6}$$

and is equal to that for the circuit in Fig. 3-14. There is no problem with the noise in output U_o if the highest frquency of the signal is lower than $1/(2\pi R_1 C)$.

$$f_h < \frac{1}{2\pi R_1 C} \tag{3-17}$$

3.5.7 Higher-Order Differentiators

Low-order differentiators ($n = 1, 2$) can be verified in a relatively simple way by combining two first-order modules. Higher-order differentiators, however, are more difficult to construct, because noise increases about 2^n times (n = order of differentiation). Therefore, if no precautions are taken to eliminate the disturbing higher frequencies of the signals, the desired signal will be buried in the noise, because higher frequencies are more strongly amplified than lower frequencies. A complicated network of electronic modules is required to prevent this. The principle of these higher-order differentiators and the arrangement (layout) of their modules is explained by a flow sheet for a differentiator of fourth order (Fig. 3-40).

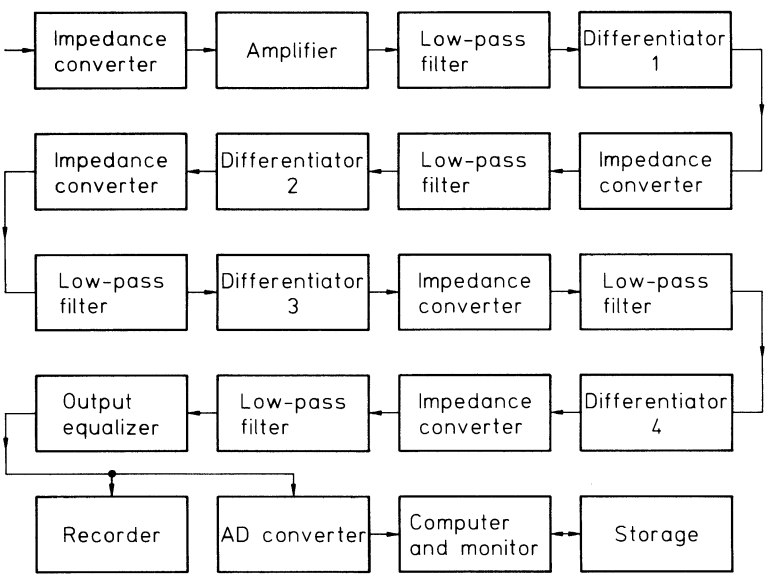

Figure 3-40. Flow sheet of a fourth-order analog differentiator.

It is important not only to avoid the differentiators' mutual influence, which is accomplished by impedance converters or optocouplers, but also to eliminate the noise of actual electric signals, which can be achieved by analog or digital filters or mathematical computation. But all these actions must lead to a compromise between smoothing the curves and distorting the shape of the signals. We studied this question thoroughly and were the first to succeed in developing analog instruments for on-line generation of low-noise higher-order derivatives up to the ninth order [42, 53–55] (see also Chapter 4).

3.5.8 Chronological Overview of Low- and Higher-Order Differentiators

From the main references concerning RC circuits [6, 32, 42, 45–48, 53–66], it can be seen that derivative analog devices have been continually used as a derivative technique for spectrophotometry (Table 3-2). Nevertheless, digital methods now predominate, perhaps as a result of the widespread use of computers in the last ten years.

Table 3-2. Chronological overview of references on RC circuits for analog differentiation of electric signals.

Author	Year	Differentiation order*	Reference
Singleton and Collier	1953	d^2	[46]
Giese and French	1955	d^2	[6]
Collier and Singleton	1956	d^2	[45]
Hariharan and Bhalla	1956	d^2	[56]
Martin	1957	d^2	[57]
Martin	1959	d^2	[48]
Collier and Panting	1959	d^2	[47]
Meister	1966	d^2	[58]
Kambara et al.	1967	d^1	[59]
Green and O'Haver	1974	d^2	[32]
Kalvoda	1975	d^2	[60]
Botton et al.	1977	d^2	[61]
Demchenko and Sandrowskii	1978	d^2	[62]
Talsky et al.	1978	d^4, d^7, d^9	53–55
Cottrell	1980	$d^2 + d^2$	[63]
Talsky	1981	d^6	[42]
Fell	1981	$d^2 + d^2 + d^2$	[64]
Zhang et al.	1984	d^2	[65]
Saakov	1987	d^2	[66]

* The indicated differentiation order is always the highest order that was generated.

3.6 Digital Differentiation

In 1953, the same year that Singleton and Collier described their analog differentiator, Morrison [67] computed first- and second-order differential curves, $\Delta i V/V$; they were obtained by substracting data points at small intervals ($\Delta V = 0.05$ eV) and are close approximations to the true derivatives. Ten curves were summated to eliminate the noise [67]. Today this method is called *point–point differentiation.* In the years, that followed up to the present, digital differentiation (using various numerical algorithms) was developed parallel to analog methods. It finally dominated over the latter owing to the evolution of computers and because of the trend to digitalize data.

We are accustomed to computation in the decimal system, but this need not be so. Computers, for instance, are based on a binary system, the binary numbers being 0 and 1. Digitalization of an analog signal is nothing more than the conversion of an electric potential to such a number. In Table 3-3, some decimal numbers are converted into digital numbers.

Table 3-3. Conversion of decimal system into digital system.

Decimal	Digital	Decimal	Digital
0	0	4	100
1	1	5	101
2	10	6	110
3	11	7	111

One of the advantages of analog differentiation is the continuous computing of the signal. No information is lost. In contrast to this, digitalization requires to be the signals quantized, that is, data is only measured at certain time intervals. In the space between two data points no information is available, and it is assumed that the two points can be connected by a straight line. But this is not always the case (Fig. 3-41). Therefore,

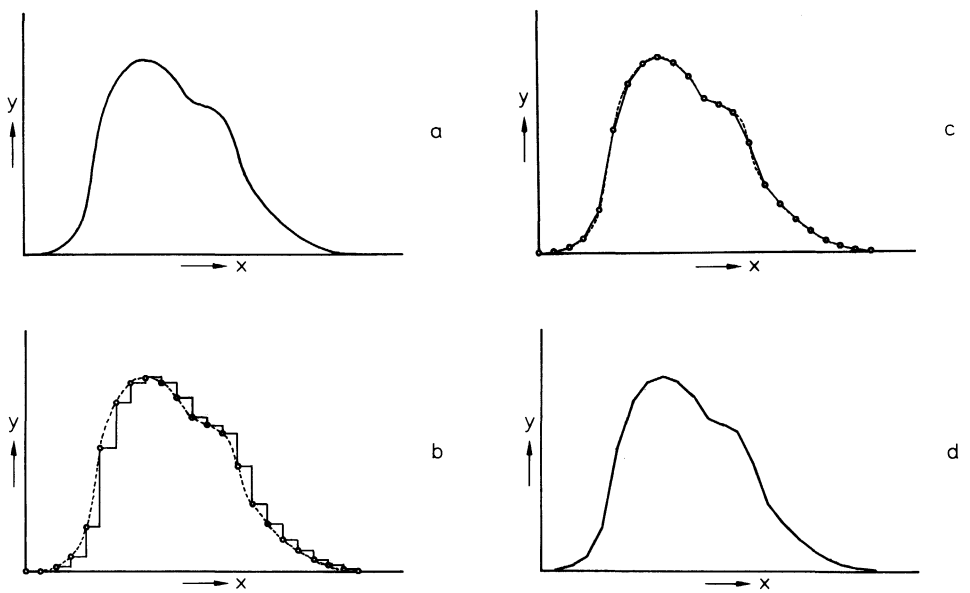

Figure 3-41. Comparison of analog and digital signals.
a) Analog signal; b) digital data points (AD converted); c) reconverted (DA-converted) signal without smoothing (——); original analog signal (------) (small deviations visible); d) reconverted analog signal. (Since it is still angular, the signal must be smoothed by a low-pass filter).

a true signal between the points could become lost; reconversion into an analog signal gives a discontinuous curve. For smoothing, analog or digital filters must be used. Of course, data points could be taken at short intervals, perhaps every few microseconds, thereby maximizing the amount of information obtained. This is true not only for the effective signal but also for the noise – which would interfere with the differentiation process. Therefore, a compromise must always be made between step width (or time interval) and number of data points on the one hand, and resolution, signal-to-noise ratio, and memory capacity on the other.

The steps necessary for digital (or numerical) differentiation are similar to those for analog differentiation. After AD conversion the effective signals have to be smoothed – or the data separated from the noise in another way –, then differentiated, and finally printed, or plotted, or analog DA converted and smoothed again (Fig. 3-42) for recording.

Depending on the circumstances, either all or only some of the modules will be necessary.

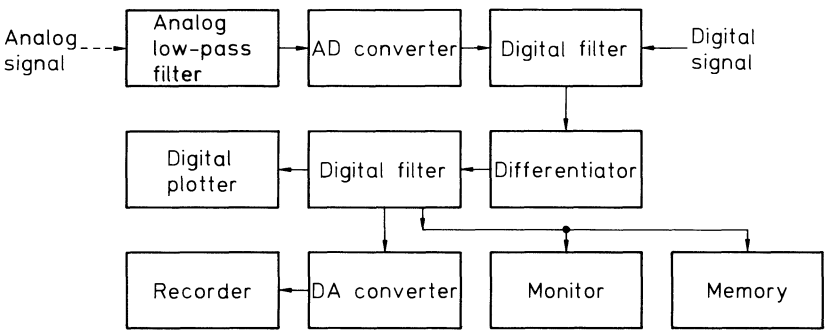

Figure 3-42. Flow sheet of a first-order digital differentiator. To get higher-order derivatives the data must be stored, and differentiated again.

3.6.1 Dual-Path Storage Oscilloscope

The *dual-path storage oscilloscope* is a device fitted with two independent analog inputs, a two-channel AD converter, a two-channel monitor, two input memories and one output memory, and a DA converter with an integrated analog filter.

For differentiation, the signal is first immediately stored in channel 1. Then the same signal is stored in channel 2 and shifted one or more millimeters (or $\Delta\lambda$). When both spectra are then subtracted from each other, the result is the first derivative, which can be plotted digitally or, after DA conversion, recorded with an analog system (see [42] and Fig. 3-5).

For higher-order differentiation, we used the following technique [42]:

With the help of an additional digital memory, d^1 is stored and then sent back twice with a difference of $\Delta\lambda$ to the storage oscilloscope. After substraction one obtains the second derivative. This process can be repeated if higher-order derivatives are desired.

In that case, after each newly generated derivative, the noise must be eliminated with a digital low-pass filter (Fig. 3-43).

Another method leading to the same results involves the use of two storage oscilloscopes. Here again, it is advisable to filter the signals after each cycle (Fig. 3-44).

A so-called *hybrid differentiator* is composed of an analog differentiator and two storage oscilloscopes. The advantage of this combination is the relatively simple analog differentiation and filtering process. On the other hand, noise increases through repeated AD–DA conversion [42] (Fig. 3-45).

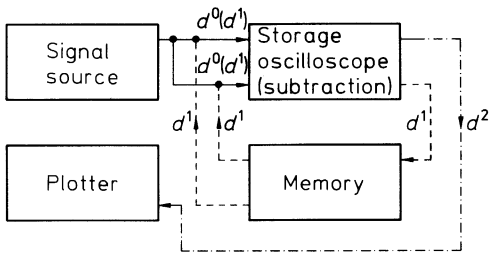

Figure 3-43. Flow sheet of a second-order differentiator, consisting of a two-channel digital storage oscilloscope and an intermediate digital memory.

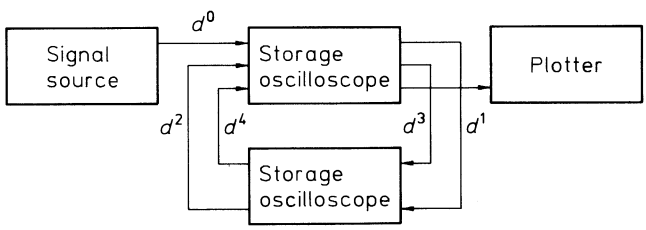

Figure 3-44. Flow sheet of a fourth-order differentiator, consisting of two two-channel digital storage oscilloscopes.

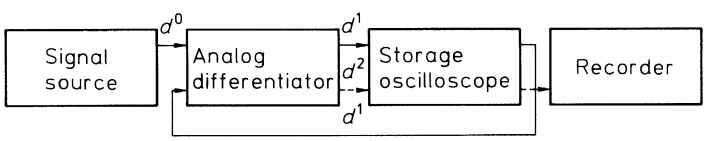

Figure 3-45. Flow sheet of a hybrid differentiator.

3.6.2 Intelligent Plotter

The *intelligent plotter* is a compact apparatus consisting of a small computer with fixed number-coded operations for the storage of AD-converted signals (up to eight analog inputs) in the integrated memory or, with the help of the integrated disk drive, on floppy disks; in addition, data manipulation is possible, e.g., by addition or subtraction of curves, multiplication, division, differentiation, integration, smoothing, logarithms, data shifting and so on. The results of these computations can be given on an integrated plotter. Furthermore, it is a useful apparatus for generating higher-order derivatives (see [136]).

3.6.3 Computers

3.6.3.1 Hardware

The core of a digital differentiator is the computer. Prices vary considerably depending on quality. Nowadays, at least a 16- or 32-bit computer with a *minimum* of 512 kB, or preferably 1 MB, internal memory (RAM), two floppy drives, and, if possible, a Winchester drive (hard disk) is required. This equipment usually provides derivatives of high quality.

Smaller or low-cost spectrophotometers have integrated computers which also allow for differentiation, aside from programming the functions of the apparatus, but they are usually only suited for low-order derivatives because it is not possible to modify the differentiation parameters, and if so, then only to a very limited extent. Of course, the operation itself is simple – only a matter of pressing a button –, but the quality of the derivatives is unsatisfactory, the signals are often very noisy, and standard deviations are very high. Spectrophotometers with separate computers are more flexible because they allow the use of more complicated software, and it is possible to adapt the program to the circumstances that are required for a particular problem. Moreover, the software can also be employed in other cases of signal resolution.

3.6.3.2 Software

The quality of the software is almost more important than the computer itself. Many programs are available, but only few are of high quality. Often, user-friendly software is too rigid with regard to alteration of derivative parameters, filtering, and smoothing. This may be the reason behind the success or failure in solving an analytical or spectroscopic problem. It is not possible to discuss in this text the complete range of available software, but we can point out the essential elements of the programs.

From previous experience, we have concluded that the software should integrate the following features:

- Control of spectrophotometer functions
- data storage with variation of time or λ steps
- signal storage
- accumulation of spectra
- accumulation and step-by-step averaging of data points (time-averaging)
- true average (arithmetic mean)
- "running window" (smoothing with variable steps and number of data points)
- linear and nonlinear regression
- least-squares procedure
- other filter functions (e.g., according to Spline or Fourier)
- point-point differential quotient
- Savitzky-Golay polynomials with variable $\Delta\lambda$ steps and filtering modes (number of points for smoothing)
- data manipulation, e.g., addition; subtraction; multiplication; division; logarithms; integration; standardization to highest peak; "window" function (i.e. the magnification of details of the spectra); and shifting of signals along the x- and y-axis

- peak-zero evaluation
- side-peak-side evaluation
- lettering of spectra
- printing and plotting of data in various dimensions.

Some of these points will be discussed below.

3.6.4 Numerical Manipulation of Digitized Curves

There are different reasons for the numerical manipulation of data. One of these is data reduction; others are filtering and smoothing, magnification of the amplitude of the spectra or of certain spectral features, and standardization. After the preceding computations, different algorithms for digital differentiation can be applied.

3.6.4.1 Smoothing and Filtering

Sliding Average

The higher the signal-to-noise ratio rises, the better the sensitivity of a measuring instrument will be. One of the simplest methods of lowering the noise is the *sliding average computation*. For this purpose an odd number $(2n + 1)$ of equidistant ordinate values is added and divided by $(2n + 1)$. The mean value of the ordinate is then assigned to the middle of the group of points, to the data point $n + 1$. Then the first point of the group is omitted, the next point is added to the other end, and the same procedure is repeated (Fig. 3-46a).

This method is easy to carry out and often sufficient, but it has one great disadvantage: Like all smoothing manipulations, it influences not only the noise but also the actual signal, which becomes distorted. In particular, sharp peaks are strongly flattened, and the resolution of overlapping signals is changed for the worse. Another disadvantage must also be considered. If computation starts at x_{-n}, but the scan of the spectrophotometer at x_0, then n data will be missing. In this case either n points at the beginning of the smoothed curve are artifacts or the curve must be deleted from x_0 to x_n. Of course it is also possible to begin scanning and computation at x_{-n}, but plotting not before x_0. The same effect occurs at the other end of the x range.

Weighted Average

The disadvantages described above can be moderated if the ordinate of the middle value and the adjoining data are more strongly weighted than the outer data of the respective group of values. This can be achieved by *triangulation smoothing* (Fig. 3-46b), for instance, or by *exponential smoothing* (Fig. 3-46c). Pavlath and Millard [68] succeeded in smoothing noisy spectra with a three-point linear calculation. Each point is calculated according to Eq. (3-18).

$$\bar{y}_n = \frac{1}{4} y_{n-1} + \frac{1}{2} y_n + \frac{1}{4} y_{n+1} \tag{3-18}$$

Generally, the smoothed ordinate $\bar{y}_i$ of the ith data point is a function of n $(=2\,m+1)$ non-smoothed equidistant points y_i and of the coefficients c_k.

$$\bar{y}_i = \frac{\sum_{k=-m}^{k=m} c_k \cdot y_{i+k}}{\sum_{k=-m}^{k=m} c_k}, \tag{3-19}$$

In the normal sliding average, c_k is a constant, and in the weighted average c_k becomes linearly or nonlinearly (exponentially) smaller.

Without any smoothing, even moderate background noise can cause a jagged structure in the derivatives, which makes any quantitative evaluation impossible. On the other hand, extensive smoothing eliminates the characteristics of some low-intensity peaks. Note: All smoothing operations will demand a compromise between SNR and resolution.

One-Sided Exponential Average

When c_k is zero and $k > 0$, *one-sided smoothing* occurs. Only previous data points are considered (Fig. 3-46d). The coefficient c_k drops exponentially with $k \leq 0$, and this digital filter has the mode of action of an RC-analog filter. Since data points in the past and in the future are treated differently, – the signal is distorted.

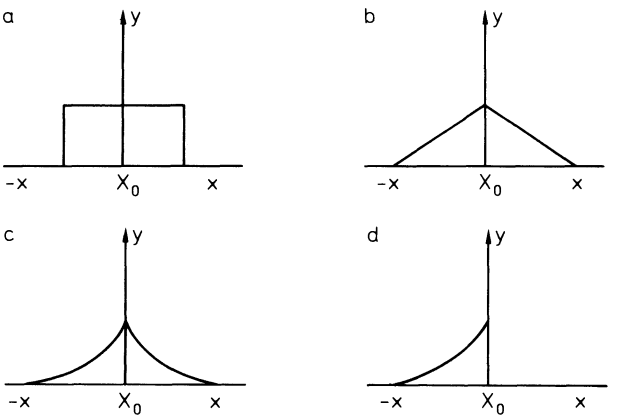

Figure 3-46. Some methods of data averaging.
a) Sliding average (symmetrical); b) triangulation averaging; c) exponential averaging; d) one-sided exponential averaging.

The coefficient of a one-sided exponential filter leads to a geometric progression. In this case the number of data points to be considered should be infinite and, therefore, the exponential filter can only be approximately realized by an "iterative filter". A more common method of realization is based on the recursive function [69, 70].

$$\bar{y}_i = (1-q)\,y_i + q \cdot \bar{y}_{i-1} = y_i + q(\bar{y}_{i-1} - y_i) \tag{3-20}$$

In order to calculate the smoothed ordinate $\bar{y}_i$, not only unsmoothed ordinate data were used but also the foregoing smoothed ordinate value $\bar{y}_{i-1}$. The greater the constant q ($0 \leq q < 1$), the more drastic the smoothing will be. To initiate the algorithm, $\bar{y}_1$ is used as y_1. The smoothing operation then proceeds stepwise. Theoretically, infinite steps are necessary for the full effect but, in practice, optimal results can be obtained in a short period of time.

Finding the algorithm with a PC program is relatively simple (Fig. 3-47); moreover, it is useful for on-line computing. The data are smoothed in real time and simultaneously reduced. No untreated data need to be stored.

In all the methods discussed in Sec. 3.6.4.1, the smoothing algorithm does indeed reduce noise. On the other hand, the signal becomes more or less distorted, the intensities are diminished, and the resolution of overlapping peaks is worsened.

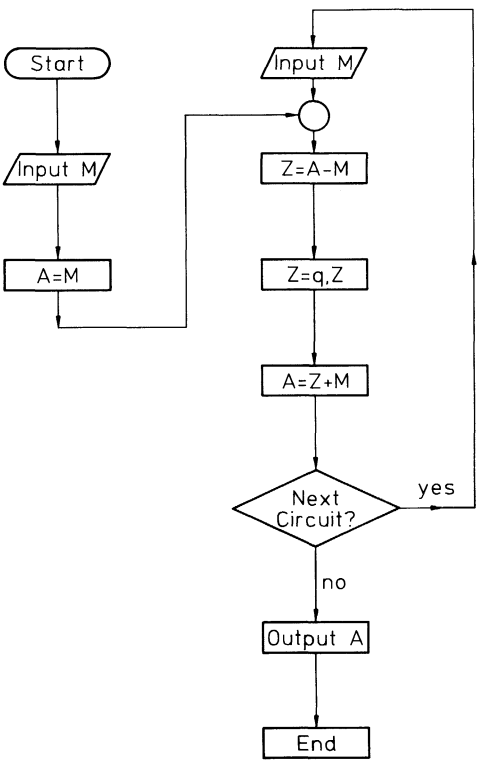

Figure 3-47. Flow sheet of a routine for one-sided exponential smoothing (see Eq. 3-20) after [70].

Smoothing by Polynomial

The simplest way to replace a series of measuring points by a well-fitted, even line is to draw it by hand. This can also be done numerically, but in that case, the criteria for computation need to be exactly defined.

a) The most common method for this purpose is the *least-square computation*. For example, a series of measuring points is to be described by a fifth-order polynomial,

$$y = a_5x^5 + a_4x^4 + a_3x^3 + a_2x^2 + a_1x + a_0 \tag{3-21}$$

The coefficient a_1 has to be estimated such that by substitution of the values of the abscissas, the second power of the differences between the computed and experimentally obtained ordinate values amounts to a minimum. Methods for estimating a_i are described in mathematics textbooks. If the a_i values for $2n + 1$ points are found, the central value, $n + 1$ $(= x_0)$, is inserted in Eq. (3-21) and a corrected y value is computed. Then the first point of the group is obmitted, the next point on the other end is added, and the procedure is repeated. Generally, for each group of points, a new set of coefficients of a_i is necessary. This method is time-consuming even though the computing speed is high. Therefore it is only useful for off-line computation.

b) Savitzky and Golay [71] simplified this smoothing procedure with a set of coefficients for computing weighted averages by approximate polynomials of second to fifth power. Eight years later Steinier et al. [72] corrected some errors in their convolution array, but fortunately, this hardly influenced the results of the smoothing computations.
Employing the smoothing function according to the Savitzky–Golay method for each measuring value of y_i, a new value of y_i is computed by a weighted average. The general form of this polynomial is

$$\bar{y}_j = \sum_{i=-m}^{i=m} \frac{c_j y_j + i}{N} \tag{3-22}$$

The subscript j denotes the original ordinate values y_j.
The width of smoothing runs from x_{j-m} through x_j to x_{j+m}. Unlike an analog RC filter, which takes only values that lie in the past, this polynomial also applies values that lie in the future. Depending on the shape of the curve, one can choose between a quadratic–cubic or quartic–quintic polynomial form. The width of smoothing (N) can be varied between 5 and 25 (only odd numbers). Similar to computing the sliding average, the weighted average also has the disadvantage of giving artifacts at the beginning and at the end of the data because in the computation of the new smoothed value, values that come before it and after it are always included. Therefore it is better to take a smoothing interval from $x_{(j-m)+m}$ through x_{j+m} to $x_{(j+m)+m}$ that is equal to $x_j \ldots x_{j+m} \ldots x_{j+2m}$. In that case, the first smoothed point is not y_j but y_{j+m}, and at the end of the data series only m points are wrong and must be omitted. The quality of SNR is not only determined by the polynomial order and width of smoothing, but also by the number of smoothing operations. The best result for any problem can therefore only be obtained by optimizing these parameters.
Generally, the efficiency of smoothing depends on the number of points used (width of smoothing). The higher this number, the lower the noise. But the signals become especially distorted if the smoothing width is broad. This is evident if the *smoothing ratio r*, the ratio of the smoothing width to the half width of a peak, is considered. For singel smoothing of a Gaussian peak, the peak height A and the peak area AR

drop down when r rises. This becomes evident when $r > 1$ for A and $r > 1.3$ for AR (Fig. 3-48a). Also, for multiple smoothing operations r is of great influence (Fig. 3-48b). If r is small (0.343), 32-fold smoothing has very little influence on the peak height ($\Delta A < 1\%$). On the other hand, if r is large (1.375), fourfold smoothing decreases A by 12%. These considerations show that, under special circumstances, the shape and intensity of the signals are only insignificantly modified by the Savitzky-Golay polynomial. Using the same number of points, the smoothing effect is less evident than by application of other smoothing algorithms. But if the smoothing width is greater than the half width ($r > 1$), the distortion rises rapidly.

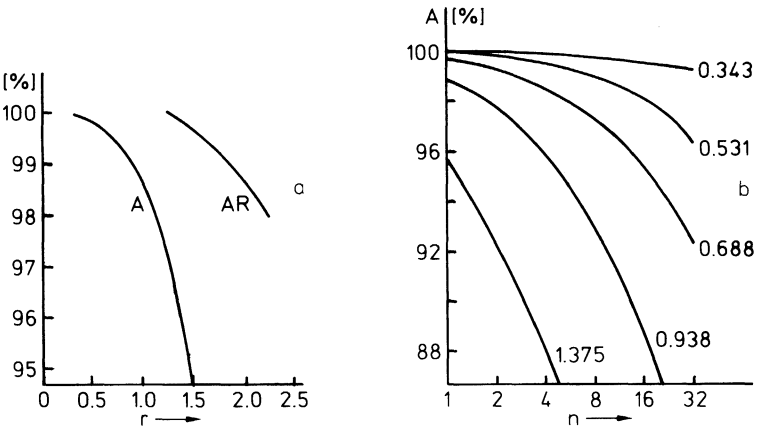

Figure 3-48. Influence of smoothing parameters.
a) Influence of smoothing ratio r, (the ratio of smoothing width to FWHM), on amplitude A and area AR, respectively, of a Gaussian band; zero order, single smoothing operation (according to [73, 81]); b) Influence of n smoothing operations on signal amplitude A at different smoothing ratios r (according to [73, 81]).

c) Kawata et al. [74] introduced an algorithm using *adaptive least-mean-squares smoothing* for minimizing the noise of spectra and other signals. This computation follows the local mean and the local variance of the observed spectra. It provides more acceptabel results for polynomial curve fitting than the convolution algorithm, and it requires less computation time. For a given data point, the Kawata method requires only five multiplications (or divisions) and seven additions (or subtractions) whereas the convolution algorithm requires $m + 1$ multiplications (or divisions) and m additions, where m is the number of weighting coefficients for each point [74].

d) Another widely used smoothing procedure is a piecewise cubic polynomial interpolation with continuous first-order derivatives at the data points. This algorithm, called the *Spline method,* employs a set of n data points. The computation leads to a maximum degree of smoothness, minimal oscillation, and minimal overshoot [75-77].

e) It is also possible to lower the noise by applying the Fourier function. If the higher Fourier coefficients are not taken into consideration, the retransformation will result in a highly filtered curve. The procedure is indeed very expensive in terms of computational effort. Furthermore, it is difficult to distinguish between the frequencies of the noise and those of the true signal because in general, they will, to some extent, overlap. Therefore, the signals are somewhat distorted [75, 76, 78]. The influence of the number of Fourier coefficients is shown in Fig. 3-49.

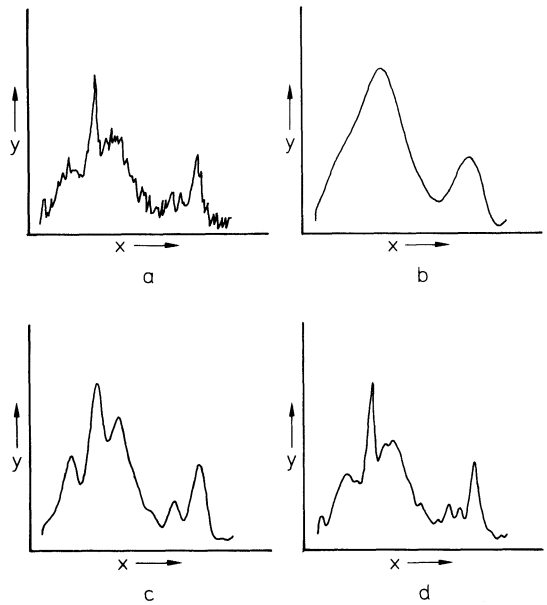

Figure 3-49. Smoothing by Fourier analysis (according to [76]).
a) Five overlapping peaks with background noise, 128 single data points; b) signal smoothing with 4 Fourier coefficients; c) with 10 Fourier coefficients; d) with 20 Fourier coefficients. (c) shows the best compromise between smoothing and loss of information.

Density and Number of Data Points

A greater density of data points taken from a continuous signal gives correspondingly more information and a more precise signal. The same holds true, though, for the noise (Fig. 3-50). On the other hand, a higher number n of points used in *polynomials* (set of

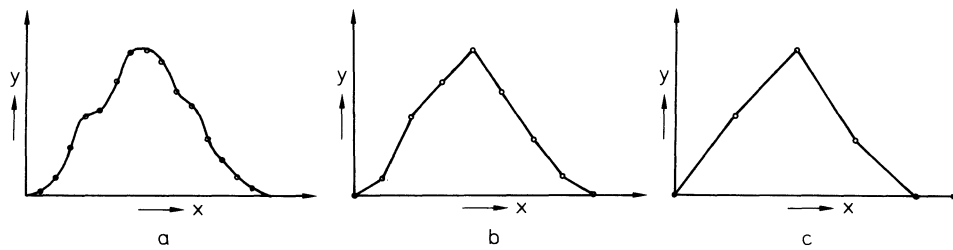

Figure 3-50. Smoothing and distortion of signals by reduction of points (schematic drawing).
a) 15 points; b) only every 4th point; c) only every 8th point (low point density).

points) increases the smoothing effect, which results in a smaller amount of information obtained.

Suppression of Spikes

In digitized spectra, single sharp peaks sometimes occur and differ from the course of the curve. These "spikes" can be traced back to disturbing impulses which are caused by bad grounding of the AD converter or by incorrect AD conversion. Smoothing procedures do not clear away such signals. On the contrary, they are sharpened and come to resemble the true signals. Therefore, they need to be eliminated before smoothing can begin. This can be done by introducing a threshold value, but care must be taken that true peaks are not eliminated as well.

Time-Averaging Procedures

The signal-to-noise ratio can also be improved by the point-by-point addition of a set of spectra. When n spectra are accumulated, the coherent amplitudes of the signals increase by a factor of n. But due to the statistical character of this procedure, the average amplitude of the incoherent noise rises only by a factor of $\sqrt{n}$ (Table 3-4 and Fig. 3-51). Strictly speaking, the $\sqrt{n}$ law is only valid if the noise can be considered "white noise", that is, if all frequencies are regularly distributed [79].

Table 3-4. Influence of time averaging on noise reduction.

n [a]	$\sqrt{n}$ [b]	% N_r [c]
5	2.23	44.6
10	3.16	33.2
20	4.47	22.3
50	7.07	14.1
100	10.00	10.0
200	14.14	7.1
500	22.36	4.5
1000	31.62	3.2

[a] n: magnification factor of the true signal by accumulation of n acans.
[b] $\sqrt{n}$: magnification factor of the random noise (white noise).
[c] % N_r: remaining noise after n accumulations.

Table 3-4 and Fig. 3-51 make evident that the dramatic effect of noise reduction drops off after about 50–70 cycles; beyond that the noise reduction is relatively low considering the amount of effort required. Therefore, a combination of time averaging and digital smoothing is often more favorable. Note that the noise also decreases by $\sqrt{n}$ when the number of points in smoothing operations increases. For this reason one comes

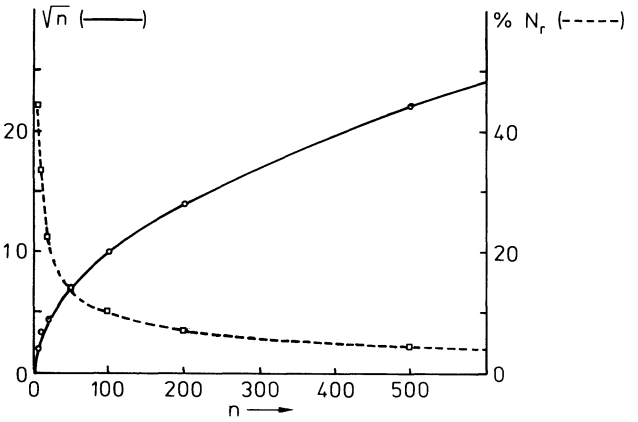

Figure 3-51. Time averaging. The solid line depicts the amplification of the random noise ($\sqrt{n}$) depending on the amplification n of the real signal by accumulation of n scans. The dashed line represents the percentage of remaining noise after accumulation of n scans, relative to the noise of the untreated signal.

practically to the same result whether data points of 100 spectra are averaged, or only 20 scans are taken and smoothed by a 5-point polynomial:

$$\sqrt{100} = \sqrt{20} \times \sqrt{5} \tag{3-23}$$

$$10 \approx 4.47 \times 2.24 = 9.995 \tag{3-24}$$

It is also possible, of course, to raise the time constant of registration by a factor of n and to average each single point. However, this *point-by-point time averaging* is time consuming, and beyond that, the smoothing effect is only seen when all points of the spectra are averaged, that is when the procedure is finished. It must also be considered that the registration time may take several hours if normal spectrophotometers are used. In this case a fast-scanning apparatus with diode arrays or vidicon detectors is preferable, because long-term stability is not always sufficient considering the high standards required.

The accumulation of signals and the division by the number of scans described above produces a true data average. In order to reduce the occupied memory space, the so-called *sliding time average* is sometimes used. Two scans are averaged, then only the next scan is added to the resulting values, and the arithmetic mean of these is taken:

a) Series of stochastic values that fluctuate, say, around true value 9.95: 9.5, 10.2, 10.7, 9.8, 10.1, 9.4.

b) The sliding average of the same series is computed in the following way:

Table 3-5. Computation of the sliding average of the series of stochastic values in point a (Eq. (3-25)).

Scan and arithm. Mean	Value
1st Scan	9.50
2nd Scan	10.20
1st Mean	9.85
3rd Scan	10.70
2nd Mean	10.28
4th Scan	9.80
3rd Mean	10.04
5th Scan	10.10
4th Mean	10.07
6th Scan	9.40
Result	9.74

c) To reduce the amount of data, it is also possible to use other combinations of groups of values to compute the arithmetic mean, but only symmetrical groups lead to the true mean; otherwise the weighted means are taken. Using the same series as in a) and b), this problem is shown in Table 3-6, and the deviations are given.

Table 3-6. Deviations of the sliding average, and the mean values of the series, distributed in different groups, from the true arithmetic mean.

Data Series[a)]	Result	Deviation [%]
a 6	9.95	± 0.0
b 6	9.73	− 2.21
c 2 + 2 + 2	9.95	± 0
d 3 + 3	9.95	± 0
e 2 + 4	9.93	− 0.2
f 4 + 2	9.90	− 0.5
g 5 + 1	9.73	−2.21
h 1 + 5	9.77	− 1.18

[a)] a: True arithmetic mean; b: sliding average; c–h: mean values of the series.

Only methods c) and d) of Table 3-6 agree with the true mean of the data series in a). In the case of asymmetrical groups, e.g., in method e, the weighted means must be used:

$$\left[\frac{1}{2}(9.5 + 10.2)\right]\frac{2}{6} = 3.283 \tag{3-25}$$

and

$$\left[\frac{1}{4}(10.7 + 9.8 + 10.1 + 9.4)\right]\frac{4}{6} = 6.666 \tag{3-26}$$

The sum of Eqs. (3-25) and (3-26): 9.949
And for method h:

$$\text{First value} \ldots 9.5 \cdot \frac{1}{6} = 1.583 \tag{3-27a}$$

$$\text{2nd to 6th value} \ldots 10.05 \cdot \frac{5}{6} = 8.366 \tag{3-27b}$$

The sum: 9.949

Correlation Functions

Smoothing by *correlation functions* has its roots in either autocorrelation of the digitized signal or in correlation with another signal (cross-correlation) [80, 85]. If $f(x)$ is the function of the curve to be investigated and $a_{(\Delta x)}$ is the auto-correlation coefficient, then

$$a_{(\Delta x)} = \int_{-\infty}^{+\infty} f(x) \cdot f(x - \Delta x)\, dx \tag{3-28}$$

This means that the curve is multiplied point by point with the same signal, shifted by Δx. Thus a statement can be made about the periodicity of the data. A relative maximum of $a_{(\Delta x)}$ always occurs when Δx is equal to one of the signal distances.

The cross-correlation coefficient

$$c = \sum f(x_i) \cdot g(x_i) \tag{3-29}$$

is the degree of conformity of two measuring curves $f(x)$ and $g(x)$. For the purpose of combining the functions, not only multiplication is suitable but also other mathematical operations. For further details and information see [80, 82–85].

Chronological Overview of Digital Smoothing

Since 1956, many papers have dealt with the problem of noise elimination in electronic signals and curves. All cited methods have advantages and disadvantages. Most data manipulations for example, distort the original shape of the spectra or measuring curves. Time averaging does not modify the shape, but it is too time consuming. Careful consideration is necessary for finding the best algorithm for a given situation. Table 3-7 gives a chronological overview of the literature.

Table 3-7. Chronological overview of references for noise elimination by digital smoothing methods.

Author	Year	Method[a)]	Reference
Morrison	1953	1	[67]
Hildebrand	1956	2	[86]
McCormick and Salvadori	1964	3	[87]
Savitzky and Golay	1964	4	[71]
Zurmühl	1965	3	[88]
Whittaker and Robinson	1966	3	[89]
Isaacson and Keller	1966	3	[90]
Draper and Smith	1966	5	[91]
Ziegler, E. and Hoffmann	1968	7	[83]
Grum et al.	1972	2	[44]
Ziegler, E.	1973	2, 6, 7, 8, 9	[70]
Ziessow	1973	10	[84]
Willson and Edwards	1976	9	[92]
Enke and Nieman	1976	2	[81]
Milano and Kwang-Yil	1977	1	[93]
Talsky et al.	1978	11	[55]
Madden	1978	4	[94]
Trott and Beynon	1979	12	[95]
Pavlath and Millard	1979	12	[68]
Bromba and Ziegler, H.	1979	4	[96]
Butler	1979	1, 3	[97]
Ziegler, H.	1981	10	[98]
Norris	1981	3	[75]
Bromba and Ziegler, H.	1981	4	[99]
Gauglitz	1981	13, 14	[100, 101]
O'Haver and Begley	1981	6	[102]
Griffiths et al.	1982	2	[103]
Talsky et al.	1982	11	[132]
O'Haver	1982	6	[104]
Bush	1983	15	[105]
Tiefenthaler	1983	16	[106]
Bromba and Ziegler, H.	1983	15	[107]
Gans and Gill	1983	13	[108]
Kawata and Minami	1984	17	[74]
Hinze and Friedrich	1984	15	[109]
Gans and Gill	1984	13	[110]
Nevius and Pardne	1984	4	[131]
Kitamura and Hozumi	1985	4	[111]
Arnold et al.	1985	8	[112]
Haubensak and Talsky	1985	11	[143]
Doerffel et al.	1986	7	[85]
Kitamura and Hozumi	1987	4	[113]
Talsky	1987	11	[114]
Talsky	1989	11	[115]

[a)] 1: Accumulation of scans (time averaging); 2: least-squares polynomial; 3: numeric algorithms; 4: shortened least-squares polynomial (according to Savitzky and Golay); 5: linear regression analysis; 6: sliding average; 7: correlation function; 8: Fourier analysis; 9: review of smoothing methods; 10: different digital filters; 11: practical experience with different modes of digital smoothing; 12: linear 3-point smoothing (averaging); 13: Spline and Splaus function; 14: polynomials; 15: comparison of different modes; 16: nonrecursive filter; 17: linear mean square.

3.6.4.2 Differentiation Algorithms

In Sec. 3.6.4.1, smoothing and filtering were treated in detail. This was necessary because these operations represent the basis for the following differentiation methods. The quality of the set of data, for instance, the SNR, determines the quality as well as the suitability of derivatives. This point must be repeatedly emphasized because it is usually responsible for unsatisfactory results and artifacts.

There are many ways to generate derivatives by numerical computations. The most important digital methods are described below.

Point-Point Differentiation

In 1953 Morrison [67] was the first to use this simple mode of differentiation by substracting ordinate data in small intervalls of equal length along the abscissa ($\Delta\lambda$ or Δt, which is proportional to $\Delta\lambda$). In reality, the *difference quotient* and not the *differential* quotient is computed, but – if the steps on the x-axis are small enough – the results are nearly the same. Using the difference quotient method, the slope of the signal can be calculated according to Equation (3-30):

$$\frac{\Delta y}{\Delta x} = \frac{f(x_0 + \Delta x) - f(x_0)}{\Delta x} \tag{3-30}$$

This can be related to the Taylor expansion:

$$y_{i+1} = \frac{1}{h}(y_{i+1} - y_i) \tag{3-31}$$

and

$$y_i = \frac{1}{2h}(y_{i+1} - y_{i-1}), \tag{3-32}$$

if

$$h = x_{i+1} - x_i \tag{3-33}$$

Similar to the smoothing width in polynomial smoothing operations, the *differentiation width* $h(= \Delta x = \Delta\lambda)$ has an influence on the noise and therefore also on the SNR. The greater the differentiation width (h), the smaller the noise and the higher the SNR (Fig. 3-52). In analogy to the smoothing ratio r, the *differentiation ratio* f can be defined as the ratio of the differentiation width to the half width of a band:

$$r = \frac{\text{SW}}{\text{FWHM}} \tag{3-34}$$

$$f = \frac{\Delta x}{\text{FWHM}} \tag{3-35}$$

SW: smoothing width (number of smoothing points)
FWHM: half width (see Sec. 2.3)

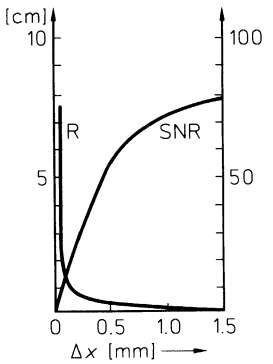

Figure 3-52. Influence of Δx (data point distance for point-point differentiation) on noise level R and SNR of a Gaussian band (A = 100 mm; FWHM = 16.65 mm); first derivative (according to [116]).

The influence of f on the peak height is stronger than the influence of r as can be seen in Fig. 3-53 a and b. If f is 0.8 ... 0.9, the peak height is reduced by about 50%. Thus the noise can be minimized by an increase in Δx; this is a simple operation. But as in all smoothing algorithms, the distortion of the signal also increases. It is therefore necessary to find a compromise between the reduction of noise and the alteration of the signal. To optimize the SNR, it is also advisable to increase the intervals of $\Delta\lambda$ with each higher derivative. Butler [97] proposed beginning with intervals of 1 nm for d^1 and intervals of 1.1, 1.2, and 1.4 nm for d^2, d^3, and d^4, respectively (see also [117, 118]).

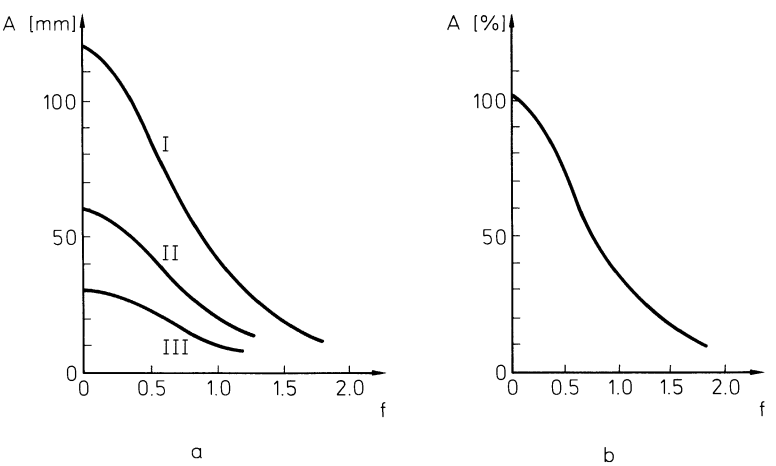

Figure 3-53. a) Influence of differentiation ratio f on peak amplitude A in the second derivative (fundamental peak: A = 100 mm; FWHM is 8.33 mm, curve I; 11.77 mm, curve II; 16.65 mm, curve III; b) change of A, expressed as percentage, versus f (according to [116]). f = Δx/FWHM.

Differentiation of Numerical Functions (Curve Data)

The principle of differentiating numerical functions is always the same. First, a polynomial (or other algorithm) is needed in order to obtain a numerical description of the curves. Then the data have to be smoothed, and, finally, the equation needs to be differentiated according to general mathematical rules. In some algorithms the smoothing operation is incorporated into differentiation and proceeds simultaneously with the latter.

Savitzky-Golay Polynomial

The *Savitzky-Golay method* is probably the most frequently used digital algorithm for generating derivatives of spectra and other curves produced by various signal sources. It is based on moving average computations (treated in Sec. 3.6.4.1, b). The best mean-square fit of a set of $2\ m + 1$ consecutive values is used for the polynomial of degree $n (n < 2\ m + 1)$, which is then numerically differentiated. Savitzky and Golay [71] tabulated the coefficients of y_i, which constitute the convolution integers for different derivatives (Table 3-8): In these coefficient tables one can choose a differentiation width between 5 and 25 data points (only odd numbers). Later, Steinier [72] corrected some minor errors in the data.

Table 3-8. Mode of convolution integers, given by Savitzky and Golay after [71].

Order of Differentiation	Order of Polynomial
d^1	quadratic
d^1	cubic/quartic
d^1	quintic/sexic
d^2	quadratic/cubic
d^2	quartic/quintic
d^3	cubic/quartic
d^3	quintic/sexic
d^4	quartic/quintic
d^5	quintic/sexic

For mathematical reasons, the beginning and the end of the differentiated data are never computed correctly, which also holds time for other polynomial algorithms, but this can easily be taken into consideration (see also Sect. 3.6.4.1) and completely compensated for. If the polynomial has a differentiation width of $n = 2\ m + 1$ points, the first and the last m of the computed new data must be deleted, i. e., $m\,\Delta\lambda$ at both the low and the high site of the spectrum. This disadvantage also exists in all smoothing operations. However, if the PP differentiation method is used, only the step width $\Delta\lambda$ at the beginning of the spectrum is lost.

The simplified least-squares procedure of Savitzky-Golay method considerably reduces the number of computations necessary, but errors may nevertheless occur in the

derivatives (e.g. shoulders as artifacts) arising from AD conversion, because the resolution of the AD converter is limited. An increment in the analog output of the spectrophotometer which is smaller than that which can be represented by the least significant increment of the AD converter will be rounded off during the conversion. Therefore it is more advantageous to smooth data *before* differentiation than afterwards; this is also valid for other digital and analog differentiation modes [111, 112]. Furthermore, the heights of the derivative signals (in comparison to those of the PP difference method) are essentially smaller. Small signals may be a great handicap, especially in higher-order derivatives, because the values must be strongly amplified – approximately 100 times (and more) per differentiation step. This causes a rise in the noise level which results in worse reproducibility of the spectra.

Other Algorithms

There are many proposals in the literature for curve fitting and digital differentiation, and the most important ones are listed in Table 3-9. Morrey [119], for example, fitted the digital data of a segment of a spectrum to a quartic equation and differentiated this convolute. A least-squares analysis of this equation gives rise to a set of linear equations. Peak positions were obtained without consideration of the type of distribution function representing the peak.

Table 3-9. Chronological overview of references to digital (numeric) differentiation.

Author	Year	Method[a)]	Derivative order	Reference
Morrison	1953	1	d^2	[67]
Savitzky and Golay	1964	2	d^5	[71]
Morrey	1968	2	d^4	[119]
Butler and Hopkins	1970	1	$4 \cdot d^1$	[117, 118]
Steinier et al.	1972	2	d^5	[72]
Horlick	1972	12	d^1, d^n	[121]
Grum et al.	1972	6, 7	d^2	[44]
Ziessow	1973	2, 12	d^2	[122]
Ziegler E.	1973	2, 11	d^2	[123]
Baun and Chamberlain	1973	9	d^1	[124]
Vandeginste	1975	2, 12	d^2	[125]
Amicon	1978	5	$4 \cdot d^1$	[126]
Butler	1979	1	d^4, d^6, d^8	[97]
Cahill	1979	1	d^2	[127]
Cahill and Padera	1979	1	d^2	[128]
Chadburn	1979	5	d^4	[129]
Gans	1980	3	d^4	[130]
Horne and Parker	1980	7, 13	d^2	[120]
Fell	1980	17	d^{10}	[35]
Talsky	1981	4	d^2, d^4	[42]
Gauglitz	1981	2, 10	d^1	[133]
Whitback	1981	8	d^2	[134]
Kaupinen et al.	1981	12, 14	d^1	[135]

Table 3-9. Continued.

Author	Year	Method[a)]	Derivative order	Reference
Talsky et al.	1982	1, 9	d^5, d^4	[136, 132]
Griffiths et al.	1982	2	d^2, d^4	[103]
Gans	1982	9	d^2	[137]
Gans and Gill	1983	2, 9	d^2, d^4	[138]
Kvaratskheli and Demin	1983	16	d^n	[139]
Heidecke et al.	1983	2, 9	d^4	[140]
AGW	1984	5	d^9	[141]
Gans and Gill	1984	8	d^2	[110]
Gartzke et al.	1984	2	d^5	[142]
Nevius and Pardue	1984	2	d^1	[131]
Haubensak and Talsky	1985	1, 2	d^6, d^4, d^6, d^9	[143]
Haubensak	1985	1	d^4, d^6	[144]
Haubensak	1985	2	d^6	[145]
Kitamura and Hozumi	1985	2	d^2	[111]
Bridge et al.	1985	14	d^x	[146]
Krivobodrow	1985	12	d^x	[147]
Sasaki et al.	1985	5	d^n	[148]
Sasaki and Inada	1986	5	d^n	[149]
Skujins	1986	2	d^4	[150]
Kitamura and Hozumi	1987	2	d^2	[113]
Talsky and Ristić-Šolajić	1987	1, 18	d^6, d^4	[151]
Talsky and Schmid	1988	2	d^8	[152]
Talsky	1989	9	d^n	[165]
Talsky and Schmid	1989	2, 15	d^4	[166]
Talsky and Ristić-Šolajić	1989	1, 15, 18	d^4	[167]
Talsky et al.	1989	1, 2, 15, 18	d^4, d^8	[168]

1: Point-Point differentiation (difference quotient); 2: simplified least-squares polynomial combined with differention (Savitzky-Golay polynomial); 3: differentiation of superposed distribution curves (curve fitting); 4: differentiation by spacially delayed curves; 5: algorithm not named; 6: Lagrangian differentiation formula; 7: differentiation of approximating polynomials; 8: differentiation of smoothened data by Spline functions; 9: comparison of different methods; 10: Splausian function; 11: Taylor expansion; 12: differentiation of Fourier transformation; 13: Chebychew filter polynomial differentiation; 14: digital differentiation of reflectance (log K/S spectra); 15: digital differentiation of reflectance (log R^{-1} spectra), using flexible optical fiber measuring heads; 16: evaluation of optimum selectivity of n^{th}-order derivatives; 17: polynomials for synthesis of Gaussian and Lorentzian distribution curves and their derivatives (even orders up to d^{10}); 18: analog-digital hybrid method

Other modes of numerical differentiations are based on Lagrangian differentiation formulas, Taylor expansions, and Spline and Splaus functions. Also, differentiation is used after discrete Fourier transformation. In this case, noise can be eliminated by neglecting the higher terms of the polynomial, but often it is not easy to find the limit of noise frequencies and the higher frequencies of the effective signals.

Last but not least, digital filter functions (e.g., of Butterworth, Chebychew or Gauss) have sometimes been used for derivative algorithms [120]. In this connection, it must be signalized that it is also possible, of course, to differentiate a sum of approximating polynomials, as was performed in the previous chapter (see Sec. 2.3.4).

References to Digital Differentiation

As already described, there are many methods of digital differentiation. Each method has its advantages and disadvantages – nevertheless all algorithms are based on approximating polynomials. Therefore, deviations of the true signals may occur and a compromise between SNR and resolution is necessary. In order to estimate the results, it is important to know which method to use for differentiation (see Table 3-9). The possibility of artifacts can then be minimized.

3.7 Vidicon and Diode Array Devices

Milano et al. [153, 154] and Cook [34] introduced an approach to derivative spectra by substituting electronic wavelength modulation for the mechanical systems used in derivative spectrometers. This effect is achieved by superimposing a low-amplitude, periodic wave form on the horizontal sweep signal. In this way d^1 spectra were generated. Warner et al. [155] applied a vidicon detector for fast detection of fluorescence spectra and obtained derivatives of the stored data by digital computation. Cook et al. [156] also made use of a silicon *vidicon detector* for multichannel operations in rapid UV-VIS spectrophotometers with the possibility of first-order differentiation. For the same purpose Milano et al. [93, 157] used a multichannel linear *photodiode array* for detection of spectra in polychromator optics and stored data manipulations (d^1). Technical explanations of the principles of diode array and vidicon devices can be found in [158-161].

3.8 Hybrid-Derivative Modules

The combination of advantages of both the analog and digital techniques results in a so-called *"hybrid module"*. In these devices the differentiation – and sometimes also the smoothing operations – are performed in analog techniques, but control of the module, setting of smoothing and differentiation parameters, correcting of shifting, and other mathematical manipulations as well as documentation and storage are performed digitally. Although digital methods are being adapted for all other analytical techniques, about 10-20% of all derivative devices are based on analog or hybrid techniques. The flow sheet in Fig. 3-54 makes this clear.

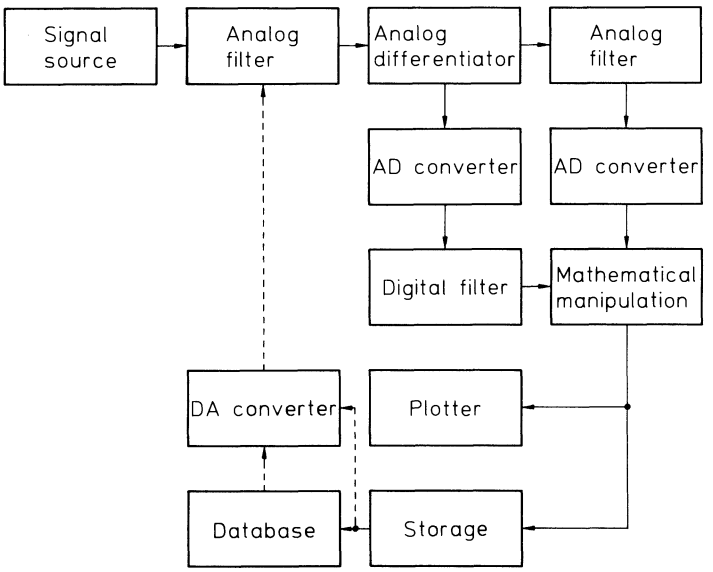

Figure 3-54. Flow sheet of a hybrid derivative module.

Theoretically, only one first-order derivative module is sufficient (even for higher orders) if the data are cycled, but because of repeated AD–DA conversion, the SNR decreases, resulting in poor quality derivatives. Therefore it is favorable to consecutively differentiate for all steps in analog mode before AD conversion takes place.

Analog filters are often used before the input of AD converters and after the output of DA converters, to lower the noise and to smooth the steps caused by digitalization.

3.9 Short- and Long-Term Storage

There are two main reasons for data storage: Measured signals can be saved for data manipulation, and the results of computation, for comparison and documentation [162, 163].

On-line devices nearly operate in a real-time mode; the results of mathematical manipulations are given on a display (monitor) or on a recorder, plotter, printer, or memory. The advantage of this procedure is that each operation can be verified immediately. In this manner, even difficult and time-consuming algorithms can be solved.

Particularly with derivative methods, great demands are made upon storage when a high reproducibility of data and a low noise level are sought, because rapid alteration of the y values with respect to the x values (that is, a steep rise of the curve) becomes

more amplified than a low alteration. This means that after differentiation high-frequency noise may predominate over the low-frequency effective signal. Then no useful evaluation is possible.

3.9.1 Analog Data

One of the simplest methods of storing analog data is to take the data down on an x, t or x, y recorder. All measured values are then continuously recorded. It is also useful to convert the analog values, (i. e., the course of signal voltage to proportional frequencies) and to store them e. g. on tape (FM method, Fig. 3-55). To recover the data, the frequencies must be reconverted to analog values. Although voltage-to-frequency (V/F) converters work with high accuracy (0.05%; 1 mV ... 1 V and 10 Hz ... 10 KHz), this method is very sensitive to variation in band velocity and also to band extension; both could cause alternations in the shape of the curves. Altogether, the accuracy of this technique is scarcely better than 1%.

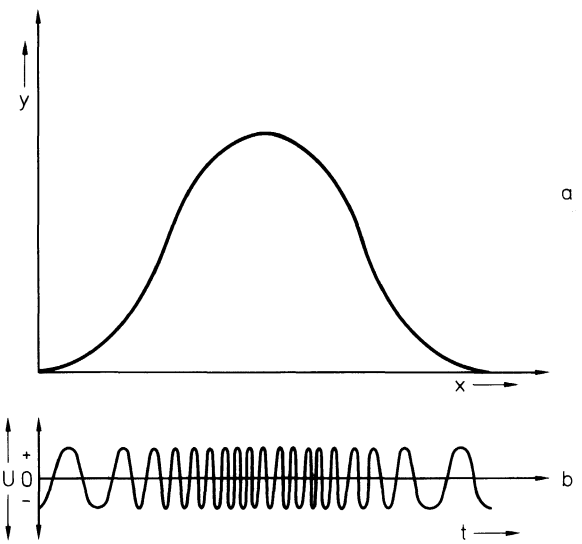

Figure 3-55. Frequency modulation technique.
a) Analog signal; b) frequency-modulated signal (qualitative graph).

Amplitude modulations that depend on voltage are also possible (AM technique) but not sufficiently sensitive for the requirements of higher-order derivative computing (Fig. 3-56). Demodulation is performed by a lock-in amplifier. Of course, the analog data can be converted to digital information, and stored in short-term or long-term memories. This is convenient if the measured values are to be manipulated (e. g., smoothed and data-reduced) or used in any kind of algorithms. Finally, the data must be reconverted by digital-to-analog conversion, and then the resulting step curve must be filtered to obtain a continuous curve. This has to be done accurately because even minute differences in the shape of the original and the DA-converted signal are amplified. In such a case, the curves will usually not be congruent.

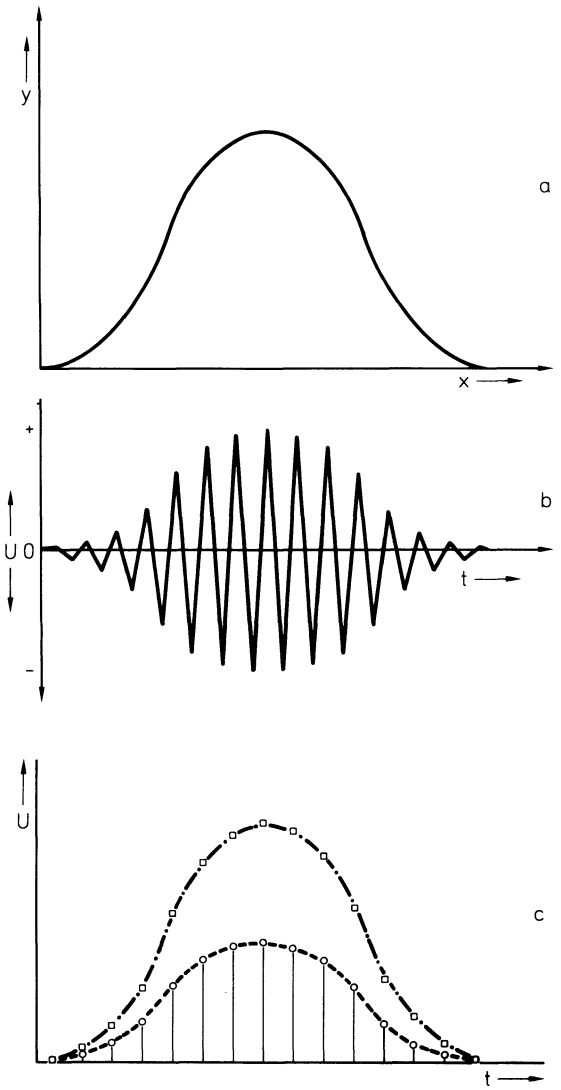

Figure 3-56. Amplitude modulation technique.
a) Analog signal; b) amplitude-modulated signal of (a);
c) demodulation by a lock-in amplifier (o--o--o) amplified by a factor of 2 (□--□--□) to bring the curve to its original height (qualitative graph).

3.9.2 Digital Data

In contrast to analog data, digital data are not continuous; instead, the data is quantized in a series of single values obtained by testing the voltage of the source at regular time intervals (Fig. 3-57). The frequency of testing should not be less than the twofold height of the Fourier component of the signal. The smaller the distance between two points, the better the curve will be described. That requires a rapid AD converter and a rapid computer. The main alternatives for data storage are given in the flow sheet in Fig. 3-58.

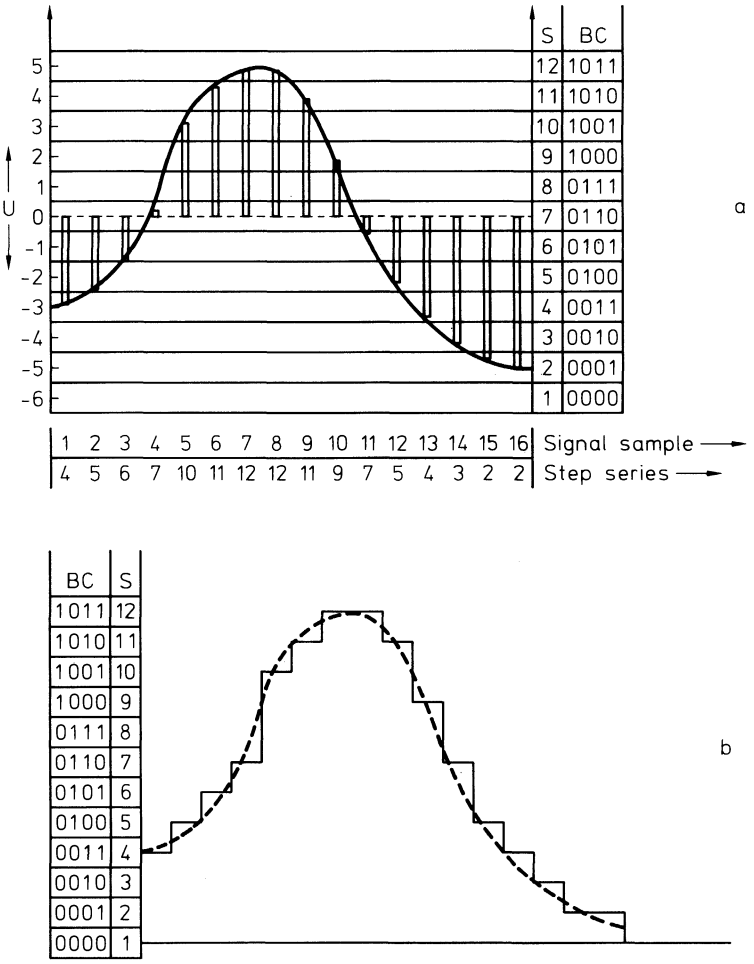

Figure 3-57. a) AD conversion with random samples (spot checks) at regular time intervals to quantize a terminally continuous signal at variable amplitudes [164]. The steps (S) of the potential (*U*) are converted into a binary code (BC). b) Reconversion of digital data to analog signals by conversion of the binary code in steps of different potentials, and smoothing by low-pass filters (the steps are much smaller in reality).

Some comments on the problem of PC modulation should be made here. Using this technique, the digitized signal is usually modulated in a pure binary code, represented as a data term (byte), that is, a bit pattern composed of a series of uniform impulses and spaces ("1" and "0"). Finally, the coded data are stored. For short-term storage, RAM memory chips and other semiconductor memories are used, which have access times from hundreds of nanoseconds to 1 microsecond. In contrast, access times for secondary long-term memories, e. g. magnetic discs, floppy discs, cassette recorders

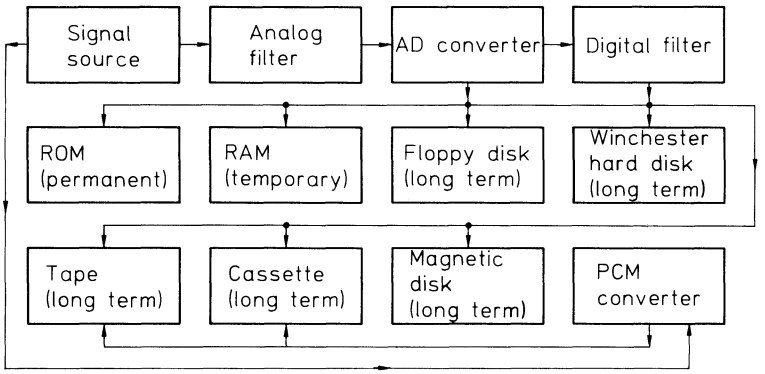

Figure 3-58. Flow sheet for possibilities of data storage. If the signal source is digital, the secound and third module must be omitted.

or tertiary stationary tape-based systems, are about 100 milliseconds and more. If the data is to be demodulated, they are converted into voltage levels, and, after smoothing, the result is a true reconstruction of the original analog signal. Advantages of this technique, which is suitable for long-term storage as well as for analog and digital data, are as follows:

- Avoidance of interference. The data terms are not damaged by interference in voltage or distortion, because at any one moment only one of two signal states is possible ("0" or "1").
- Self-timing. The PCM bit stream has its own time information coded. To recognize the state of the signal, at least a half-bit period is available. Therefore PCM signals are largely independent of their transfer velocity and transfer variations (about 20% show no interference). In contrast to FM and AM modulation, simple tape recorders can be used.
- Precision. The precision of information depends on the number of possible steps of the amplitude. In the PC modulation 8, 12, or 16 bits are used; these allow for 256, 4096, or 65536 possible steps (2^n bits!). That means 0.39, 0.025, or 0.0015% precision with respect to the full scale.
- EDP (Electronic data processing). PCM data are suited for application in computerized systems.
- Time multiplexing. Generally, the recording of a data term requires only a fraction of the time base. Therefore it is useful to record (independently) several channels of information on only one tape track.

The detailed treatment of the instrumentation for multi-differentiation of spectra and other curves should provide the necessary background so that practical comments and instructions can be given to those intending to make use of higher-order derivatives for curve analysis.

3.10 References to Chapter 3

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4 Practical Aspects

In Chapter 2 theoretical aspects of derivative spectrophotometry were treated, and in Chapter 3 the instrumentation for generation of derivatives was described. In this Chapter the sixteen years of practical experience in derivative spectrophotometry accumulated by myself and my coworkers shall be reviewed. It is hoped that our suggestions initiate the reader to adopt this very interesting and efficient analytical technique and to avoid making errors or falsely interpretating results.

An important initial question is whether the derivative technique is applicable to a particular problem. In general, it should always be used in cases where a curve contains shoulders and other nonresolved regions which have their origin in overlapping of signals. In addition, the *origin* of the data is not important, but curve analysis by multidifferentiation is primarily employed in UV/VIS spectra, because they are mostly flat and not well marked.

There are some fields in which the derivative technique was employed very successfully [1–5]. Details shall be given in Chapter 5.

Spectroscopy

a) UV, VIS, IR, fluorescence spectrophotometry; ESR, AAS, and NMR spectroscopy.
   - enhancement of spectroscopic quantitative analysis (1 to 3 orders of magnitude more sensitive; trace analysis)
   - identification of samples by finger prints
   - purity tests
   - elimination of unwanted background
   - sharpening of signals and thereby separation of two and more components (multicomponent analysis)
   - analysis of turbid solutions, suspensions, and emulsions
   - solids (powders, transparent and opaque samples)
   - gases and vapors.

Nonspectroscopic applications

a) column-liquid chromatography and gas chromatography
   - resolution of shoulders and inflection points
   - purity and identification of peaks

Derivative Spectrophotometry. By Gerhard Talsky

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b) paper and thin-layer chromatography
   - identification of spots
   - quantitative analysis

c) nondestructive investigations of surfaces of solids by reflection derivatives

d) potentiometry
   - estimation of inflection points

e) differential thermoanalysis
   - fine-resolution of temperature profiles.

Another important question is what to do to make use of derivatives. In the following, the way to generate low-order, and especially higher-order, derivatives shall be explained in a step-by-step manner. A general overview can be seen in Fig. 4-1. In this flow chart, only analog and digital modes are borne in mind, because all the other possible ways to obtain derivatives are only currently in use in some special cases. Some references to theoretical and practical aspects of derivative techniques can be found in [1-19] and in Chapter 3 the papers [31, 32, 35, 63].

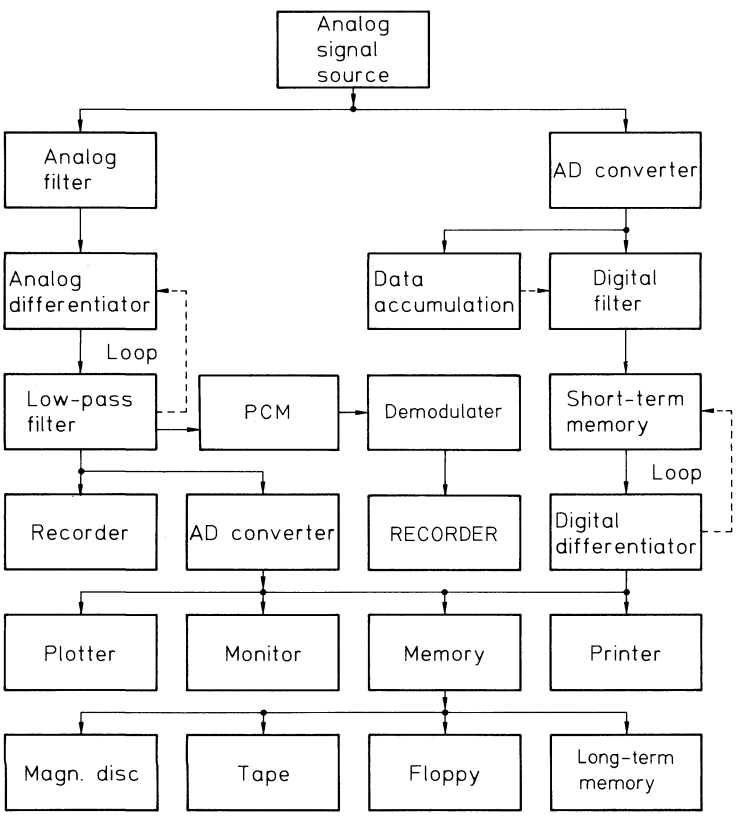


Figure 4-1. General flow sheet for analog and digital generation of derivatives. (PCM; pulse-code modulator.)

If in the next sections any experimental data for generation of spectra are given, the following abbreviations are used: mode of differentiation: MD; A: analog; D: digital; H: hybrid; S-G: Savitzky-Golay; PP: point-point differentiation.
MD: D (S-G 11) corr. means for example: digital differentiation according Savitzky-Golay 11 point polynomial; shifting of the signal ($\Delta\lambda$) corrected.

4.1 Data Source

"Prevention is the best medicine" – adapted to chemistry this proverb means that a purer educt provides a better product. Transferred to our problem, the lower the noise, the higher the signal-to-noise ratio (SNR), and thus the better the reproducibility of the derivatives. It is of prime importance for the success in generating derivatives that the data source have only a small number of interfering frequencies. Without doubt, it is possible to smooth the measuring values by analog or digital filters, but there is always the danger of distorting the signal shape. For this reason, one should analyze the outgoing signals by an oscilloscope and, if possible, determine the frequencies of the noise (i.e., mainly 50 Hz with overtones of 100 and 200 Hz).

What kind of spectrophotometer should be used as a signal-source? A double-beam apparatus has to be employed if the solvent or another component alters its absorption during measuring time. If this is not the case, a single beam optic may be preferable, because the intensity of the light source is higher, and in newly developed models the fluctuation of the lamp is very low. The latter device is affected by self-controlled feedback between intensity and lamp voltage. In a simple way the semiconductor technique allows the characteristic spectrum of the light source to be subtracted from the total spectrum. In this case it may not be forgotten that the blank determination represents only a "flash situation" of the noise spectrum and, therefore, it is not applicable for the whole operation time without taking an average of a greater number of measurements (scans).

For some years now diode-array spectrophotometers have been commercially available. They have the enormous advantage of measuring the full spectrum in a matter of milliseconds, but they still have poor resolution (about 0.5 to 2 nm only). Therefore it is necessary to bear in mind the circumstances surrounding each problem in order to find the best solution.

4.2 On-Line and Off-Line Mode

Generally, analog computations are on-line operations. This may sometimes be preferred to off-line operations. The results are simultaneously recorded and can be immediately controlled. The influence of any modification of the parameters can be registered at

once. In contrast to the off-line mode, repetition of the procedure can only be carried out by intermediate storage or by rerecording, which is not always practicable.

On the other hand, digital signal manipulations generally require off-line computation. Indeed, AD conversion takes place very rapidly (in some microseconds), and in addition, the monitoring runs at a high velocity. However, other mathematical manipulations are time consuming and require some seconds or even minutes. The advantage of this technique is the possibility, starting with only one single record stored in the memory, to optimize the parameters of the algorithm under identical conditions.

4.3 Standard Curves

In considering whether to use or buy an apparatus which has integrated a derivative device, or which is fitted with an analog device or connected with a computer and the suitable software, it is crucial to check the quality of the results *before* making a decision. Unfortunately, not all derivative modules or all softwares offered are specially suited for generating higher-order derivatives of high quality.

Sometimes the SNR of the second derivative is already so unsatisfactory (≤ 10, that means a noise level of 10% or higher) that it is advisable to make a quantitative evaluation. This hinders the application and propagation of this technique of signal deconvolution, which is firmly established not only in the field of spectroscopy and others as well.

4.3.1 Optical Glass Filters

Often manufacturers and salespeople demonstrate the effectivity of derivative devices on spectra of holmium glass filters by taking the steepest peaks. Frankly speaking, this test is meaningless, because differentiation of steep peaks is unnecessary; the peaks are resolved sufficiently. Therefore, for checking the resolution potential the holmium peak between 430 and 470 nm (λ_{max} 447.0, Fig. 4-2a) is not a valid example. But the maxima at 418.5, 537.5 or 636.5 nm (Fig. 4-2b, c and d) can be used. The signal at 447 nm may only be taken to estimate the $\Delta\lambda$ shift.

In addition to the above-mentioned filter, spectra of photographic glass filters (dark blue or light and dark green) are also suited for testing the resolution of derivative modules (Fig. 4-3). In this form, the combination of two filters is also qualified for this purpose.

The small deviation from the homogeneous course in the fundamental curve (Fig. 4-3a) can be resolved by taking the fourth derivative (Fig. 4-3b).

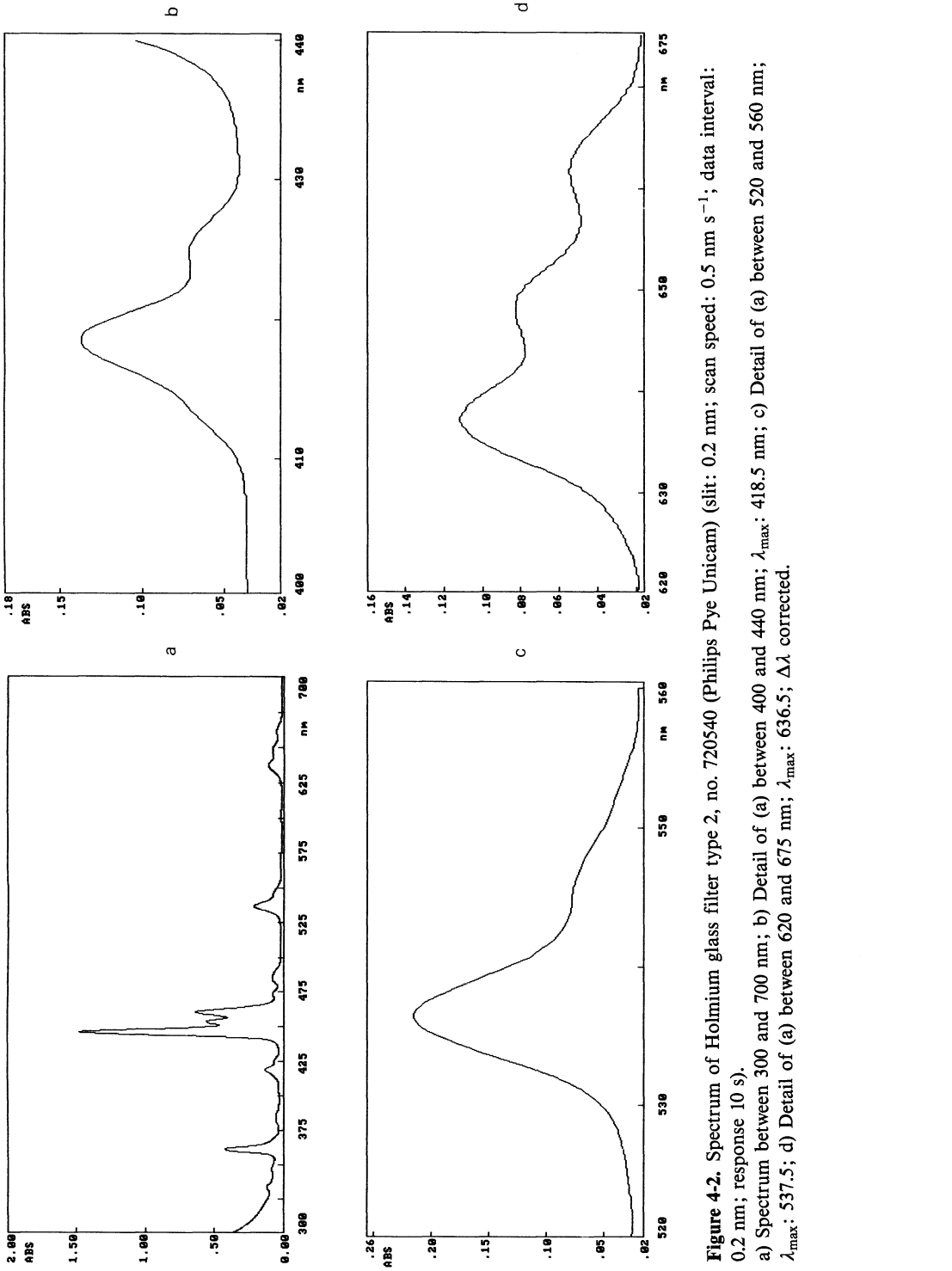


Figure 4-2. Spectrum of Holmium glass filter type 2, no. 720540 (Philips Pye Unicam) (slit: 0.2 nm; scan speed: 0.5 nm s^{-1}; data interval: 0.2 nm; response 10 s).
a) Spectrum between 300 and 700 nm; b) Detail of (a) between 400 and 440 nm; λ_{max}: 418.5 nm; c) Detail of (a) between 520 and 560 nm; λ_{max}: 537.5; d) Detail of (a) between 620 and 675 nm; λ_{max}: 636.5; $\Delta\lambda$ corrected.

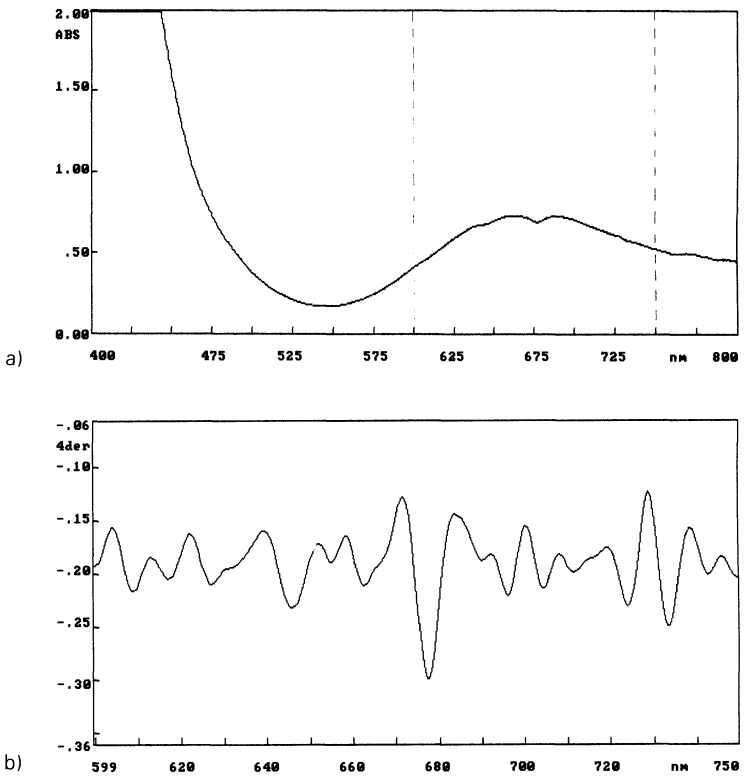


Figure 4-3. Spectrum of Heliopan glass filter (dark green) (slit: 0.2 nm; scan speed: 0.5 nm; data interval: 0.5 nm; response: 10 s).
a) Fundamental spectrum; b) fourth derivative of the detail between 600 and 750 nm. MD: D (S-G 11), corrected.

4.3.2 Solutions of Inorganic or Organic Substances

In the process of studying higher-order derivatives, four substances proved especially suitable for testing the quality of derivative devices.

Type I: $NiCl_2 \cdot 6\ H_2O$ (4% w/w in water, i.e., 40 g L^{-1}).

The spectrum of this substance has the shape of an asymmetric Gaussian curve. Only efficient derivative modules are able to resolve the signal in d^4 into two main peaks (Fig. 4-4).

Type II: Congo Red (0.002% in water).

This is a good example of a Gaussian curve superposed by an underlying exponential function, which is eliminated by higher-order differentiation (Fig. 4-5).

Type III: Methylene Blue (0.01 mg mL^{-1}).

In this case, the resolving of a shoulder on the side of a peak can be demonstrated (Fig. 4-6).

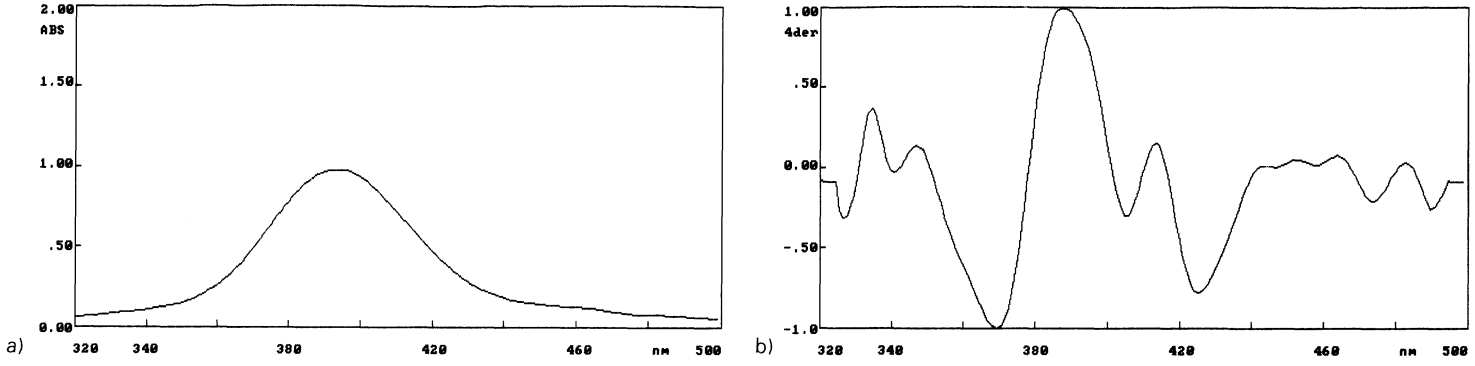


Figure 4-4. $NiCl_2 \cdot 6\ H_2O$. Conc. 40 g L^{-1} (in water) (slit: 1.0 nm; scan speed: 1.0 nm s^{-1}; data interval: 1.0 nm; response: 10 s), corrected.
a) Fundamental spectrum; b) fourth derivative; MD: D (S-G 11).

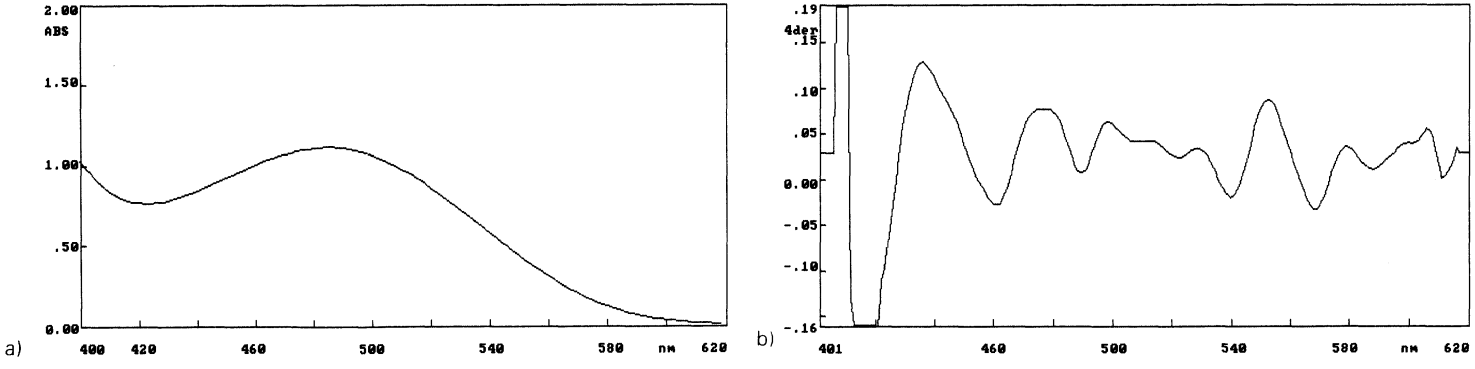


Figure 4-5. Congo Red. Conc. 20 mg L^{-1} (in water) (slit: 2 nm; scan speed: 2 nm s^{-1}; data interval: 2 nm; response: 20 s).
a) Fundamental spectrum; b) fourth derivative; MD: D (S-G 11), corrected.

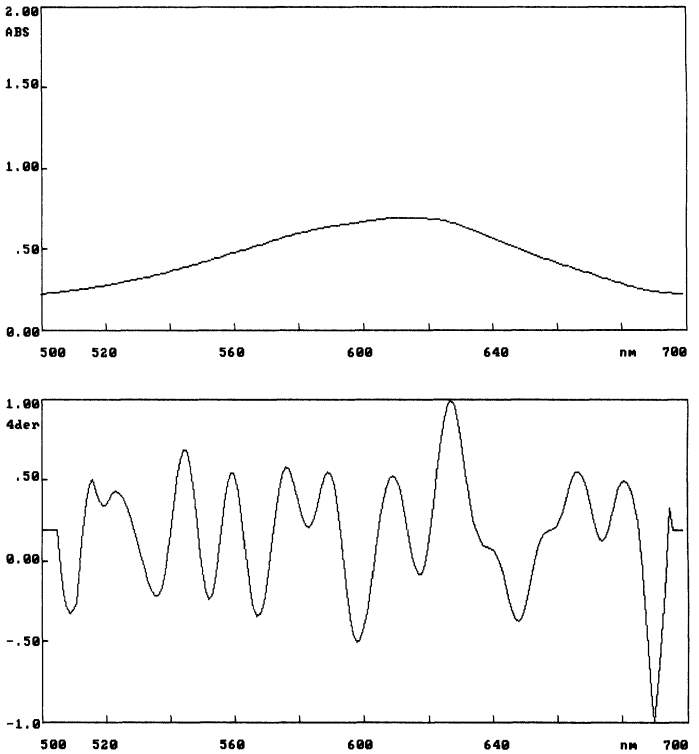


Figure 4-6. Methylene Blue. Conc. 10^{-2} g L^{-1} (in water) (slit: 1.0 nm; scan speed: 1.0 nm s^{-1}; data interval: 1.0 nm; response: 10 s).
a) Fundamental curve; b) fourth derivative; MD: D (S-G 11), corrected.

Type IV: ribonuclease A

The derivative spectra of this protein (extracted from bovine pancreas) show the resolution of small irregularities on the flanks of the fundamental spectrum (Fig. 4-7); compare also the RNase spectra in [2], generated by analog differentiation.

A selection of additional substances tested for suitability in generating differently shaped standard spectra are listed in Table 4-1.

As a first approximation, it can be stated that a higher ratio of the peak height to the FWHM-value makes differentiation correspondingly easier. This means that the order of the derivative of a small, but steep, original peak can be higher than that of an original high peak with a lower ratio. This is demonstrated in Fig. 4-8a and Fig. 4-8b. The data for this spectrum, a solution of $Ho(NO)_3 \cdot 5\ H_2O$, are summarized in Table 4-2 (cf. peak at 301 nm with the others). Deviations from this general statement may be caused by the shape of the peak.

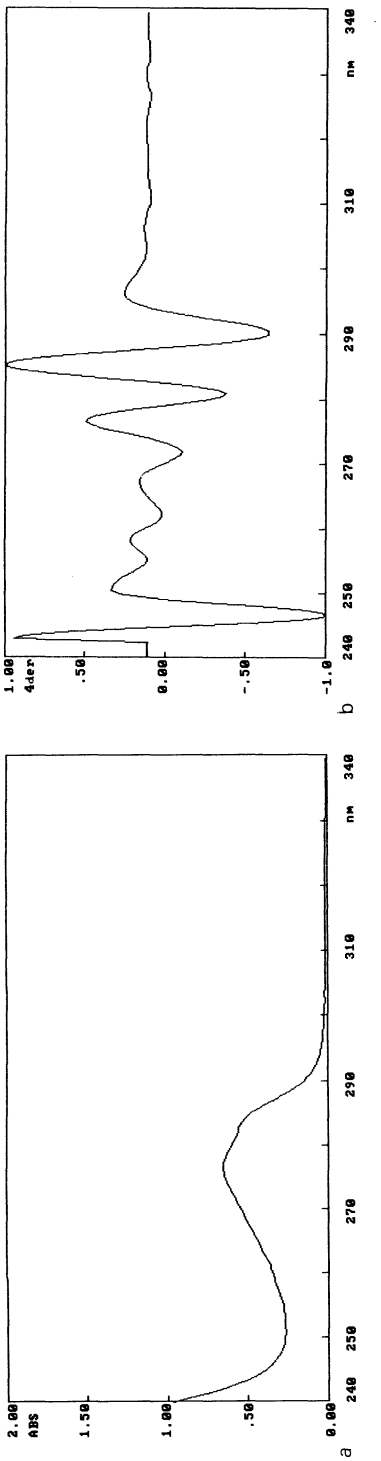


Figure 4-7. Bovine Ribonuclease A. Conc. 1 g L^{-1} (in water) (slit: 0.5 nm; scan speed: 1.0 nm s^{-1}; data interval: 0.5 nm; response 2 s).
a) Fundamental spectrum; b) fourth derivative; MD: D (S–G 11), corrected.

Figure 4-8. $Ho(NO_3)_3 \cdot 5\ H_2O$; 10 g L^{-1} (in water) (slit: 1 nm; scan: 1 nm s^{-1}; data interval: 1 nm; response: 2 s).
a) Fundamental spectrum; b) fourth derivative; MD: D (S–G 11), corrected.

Table 4-1. Substances qualified for standard spectra of various typical shapes (solutions in bi-destilled water).

Type	Substance[a)]	Conc. g/l	λ-Region	λ_{max}
I	$CuSO_4 \cdot 5H_2O$	20	550–890	779
	$NiCl_2 \cdot 6H_2O$	40	300–480	394
	$Ni(NO_3)_2 \cdot 6H_2O$	40	340–450	391
	$Co(NO_3)_2 \cdot 6H_2O$	40	270–350	294
	KNO_3	10	260–350	301
	Congo Red	0.01	280–400	341
II	$La(NO_3)_3 \cdot 6H_2O$	20	260–350	301
	$Ce(NO_3)_3 \cdot 6H_2O$	0.01	190–350	252
	KI	0.01	210–260	226
	Amido Black B	0.01	480–720	618
	Congo Red	0.01	400–620	497
III	$NiCl_2 \cdot 6H_2O$	40	550–800	715
	$Ni(NO_3)_2 \cdot 6H_2O$	80	550–850	714
	$Ho(NO_3)_3 \cdot 5H_2O$	40	520–560	537
	$Nd(NO_3)_3 \cdot 5H_2O$	40	490–540	523
	$CoCl_2 \cdot 6H_2O$	40	370–600	510
	$Co(NO_3)_2 \cdot 6H_2O$	40	400–600	509
	Methylene Blue	0.01	520–740	664
IV	$KMnO_4$	0.05	430–610	525
	$CeCl_3 \cdot 7H_2O$	1.0	190–300	253 (240) (223) (212)
	BSA	1.0	240–320	277
	CH	0.5	240–320	280
	CHG	0.5	240–320	280
	RNase	1.0	240–320	277

[a)] CH: chymotrypsin; CHG: chymotrypsinogen; RNase: ribonuclease.

Table 4-2. Data to Fig. 4-8a and Fig. 4-8b.[a]

λ-Region	λ_{max}	$H(d^0)$/FWHM[b]	$H(d^4)$ relative
620–670	641	3.6	29
520–560	537	7.2	44
475–495	486	1.6	13
440–460	452	5.7	44
410–430	417	5.0	23
350–370	362	3.0	19
250–350	301	4.4	14

[a] $Ho(NO_3)_3 \cdot 5H_2O$, conc. 10 g L^{-1} in water;
[b] H: peak height

4.3.3 Generation of Electronic Standard Curves

In Secs. 4.3.1 and 4.3.2, standard curves were optically generated by taking spectra of glass filters or solutions of certain substances. Another way is to produce a desired curve either by electronic devices or numerically by polynomials or other algorithms (cf. Section 2.3.4). By these methods, reproducible signals of high precision can be produced without a spectrophotometer. This may often be an advantage, especially when new derivative modules are invented or new algorithms investigated.

4.3.3.1 Curve Scanner

For reactivation of all values of any curves, drawn by hand or scanned by a recorder, the pen of the recorder is substituted by a sensitive photocell which follows the course of the curves with high accuracy (0.1 %), if they are not steeper than 85 °, and picks off the data which become converted into analog or digital values for further calculations [13]. This practice has the advantage of allowing for subsequent computer processing of already available conventionally recorded data. Of course, it is also possible to explore the curve by an electron beam and to digitalize the data.

4.3.3.2 Function Generators for Analog Data

Another way to produce standard signals, which can be repeated at any time as often as desired, is the application of electronic devices. The simplest instrument of this type generates square waves, triangular waves, and sine waves (Fig. 4-9).

Another possibility consists of a *bistable multivibrator* ("flip-flop"), which is time controlled by a succession of pulses, and which gives a square wave. If the latter signal is integrated by an operational amplifier working as an integrator a triangular waveform results. Finally, a sine wave is obtained if the triangular wave is modulated by a shaping circuit, consisting of a combination of resistors and diodes. In order to realize any well-defined mathematical function by approximation, a more complicated network of diodes, resistors, and capacitors are connected in series to the oscillator. The character-

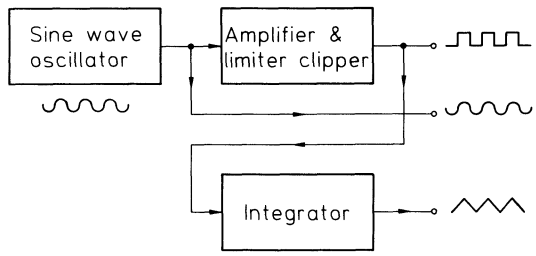


Figure 4-9. Flow sheet of a simple function generator (after [20]).

istic feature of these circuits consists of a polygonal graph of tangential or secantial segments or bended curves (Fig. 4-10 and 4-11).

In practice, a more sophisticated diode function generator must have at least eight diode steps to give satisfactory results. A modified sine wave is mostly used for the input source.

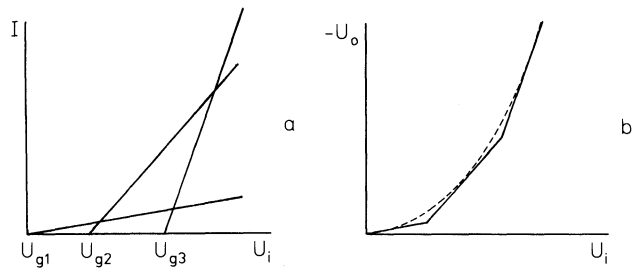


Figure 4-10. Three-Diode function generator with two breaks (schematic drawing). a) Single characteristic curves of diodes with different breaks; b) desired function (-----) and approximated signal. *I:* current, U_i: input voltage, U_o: output voltage; U_{g1}, U_{g2}, U_{g3}: break voltage. (U_i has a negative sign if the IC in Fig. 4-11 is used in an inverting network.)

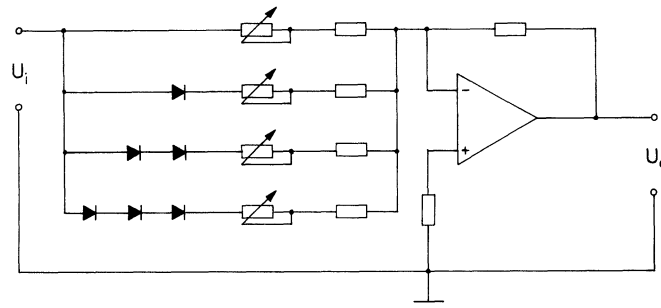


Figure 4-11. A three-stage diode function network (after [21]). U_i increases continuously, verified, e.g., by a saw-tooth generator. The resulting function is similar to Fig. 4-10, but has three breaks.

4.3.3.3 The Digital Storage Oscilloscope and other Devices

A storage oscilloscope with a variable voltage source on the input makes it possible to "write" any curves on the screen and to store them for subsequent mathematical manipulations. More effort is involved in the scanning of a cut-out template curve or a curve plotted by an electron beam (cathode ray).

4.3.3.4 Digitally Computed Curves

Finally, there is the possibility to simulate an experimental curve (spectrum) by a mathematical algorithm, e.g., by a polynomial, a Fourier transform expression, or the superposition of Gaussian or other suitable distribution curves (cf. Sec. 2.3.4, Eq. (2-41)-(2-47)). In this case, one must keep in mind that for simulation of "real" spectra it is also necessary to add a noise function, produced by a random generator, to the PC-computed curve. Otherwise, it is not possible to transfer the results of the investigations to "real" signals produced by any apparatus. Of course, it is much easier to get useful derivatives from undisturbed curves than from real spectra containing noise.

4.4 Filtering, Smoothing, and Averaging

As already mentioned, the higher-frequency noise is more strongly amplified by differentiation than the low-frequency effective signal. In order to avoid artifacts it is by all means essential to eliminate this interference with the help of analog or digital methods.

4.4.1 Analog Filtering

It is always advisible to filter out the noise, e.g., noise caused by rectification, by means of a 30 Hz fourth-order analog low-pass filter of the Butterworth type. This is also recommended if analog signals are to be AD converted.

Still lower frequencies coming from other sources can be cut off by analog filters with cut-off frequencies between 10 and 0.5 Hz, but it must be carried out very carefully and stepwise to hold the change of the signal shape in tolerable limits. This is demonstrated in Table 4-3 and Fig. 4-12.

In the example above, it can be seen that the signal height decreases significantly if τ is higher than 22 ms and becomes distorted and shifted if τ becomes higher than 235 ms (Fig. 4-12).

Table 4-3. Influence of the filter time constant τ on the height and FWHM of a Gaussian peak [14, 15].[a)]

Curve	R KΩ	C μF	τ ms	A mm	FWHM mm	Δ mm
1	–	–		100.0	21.5	0.0
2	1	0.47	0.47	99.3	21.5	0.1
3	5	1.0	5.0	96.4	21.5	0.2
4	10	2.2	22.0	94.2	21.5	0.4
5	50	4.7	235	76.8	25.0	2.0
6	100	10.0	1000	47.8	29.5	7.0

[a)] *R:* resistance; *C:* capacitance; *A:* peak height (without filtering, A is 100 mm); FWHM: full width at half maximum amplitude; Δ: shift, (seen from left to right).

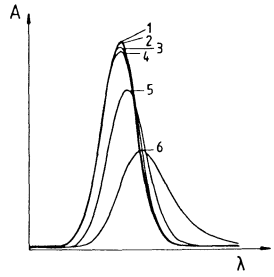


Figure 4-12. Influence of the filter time constant on the shape of a Gaussian band [10, 14, 15]. 1: $\tau = 0$; 2: $\tau = 0.47$ ms; 3: $\tau = 5$ ms; 4: $\tau = 22$ ms; 5: $\tau = 235$ ms; 6: $\tau = 1000$ ms (for further data see Table 4-3).

Experimental analog filters tend to skew the input function more or less toward longer times (in the direction of scanning). This is the price paid for an improved signal-to-noise ratio (SNR). For optimization of smoothing, one should start with the lowest possible time constant of the device and observe both the SNR and the attenuation as well as the λ shifting of the main maximum ($\Delta\lambda$). The latter is particularly important if the true position of the extrema is desired.

Based on Eq. 3-7 in Sec. 3.5.3, the cut-off frequency f_c can be calculated easily.

$$f_c = \frac{1}{2\pi RC} = \frac{1}{2\pi\tau} = 0.159\,\frac{1}{\tau}\left[\frac{1}{\Omega\,\mathrm{F}}\right]. \tag{4-1}$$

Some data are given in Table 4-4.

The ratio of R to C influences the attenuation and the behavior of the filter (filter characteristics).

Table 4-4. Relation between time constant τ and cutoff frequency f_c of the filter.

τ[s]	f_c[Hz]
10^{-3}	159
10^{-2}	15.9
10^{-1}	1.59
1	0.159

4.4.2 Digital Filtering

As a rule analog filters are connected on line with the signal source. Digital filtering takes place after AD conversion and storage of the data; it is seldom possible to work up the data source immediately. They should thus be stored temporarily on the PC-integrated RAM or permanently on a discette or on tape.

4.4.2.1 Smoothing

If only a single measurement is available or only a few scans can be made, smoothing operations must be carried out. In this case the shape of the signals becomes more or less distorted, and one must enter into a compromise between resolution and alteration of the shape. Fortunately, alteration is not important if derivatives are used for quantitative measurement and the conditions of differentiation are held constant, because only the amplitudes are necessary for evaluation rather than the true λ position of the extrema. Moreover, the standard line, or generally stated the standard curve, corrects possible deviations from the linearity of the peak height to the quantity of the components.

The most common digital filtering polynomial methods, according to Savitzky and Golay [22, 23], involve a shortened least-square computation using a "sliding window" with variable data points but other algorithms are also sometimes practicable.

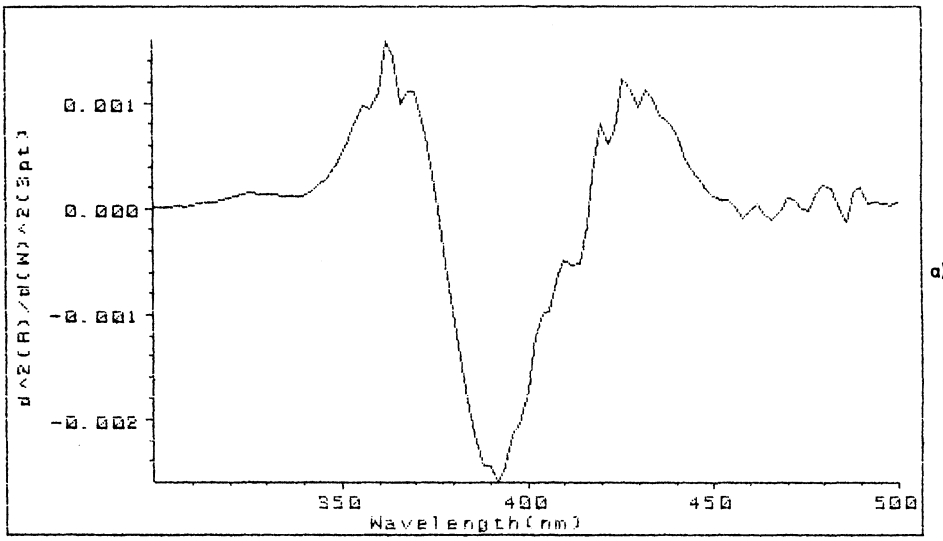
Smoothing by Fourier transform filtering is more arduous with respect to programming computation and time, but this algorithm is now being used time by time.

4.4.2.2 Averaging

The best solution of the problem of digital filtering may be *true arithmetic time averaging* of as many independent scans (or other signals) as possible. A minimum of eight accumulated scans are recommended. If the SNR is small, then 32, 64, or more scans are necessary. The optimal number can be determined by independent repetition of this procedure. If the results are congruent, the best solution is given.

Time averaging is symmetrical in time, which means that it will not induce asymmetry into an otherwise symmetrical input signal. Also, the peak height is not necessarily altered. The same is valid for pointwise time averaging, that is, the determination of the arithmetic mean of accumulated data during a given time interval at constant λ. After this step the scan continues with a definite increment $\Delta\lambda$, accumulation starts at $\lambda + \Delta\lambda$, etc. Both methods are applicable if the samples are stable and not damaged by radiation.

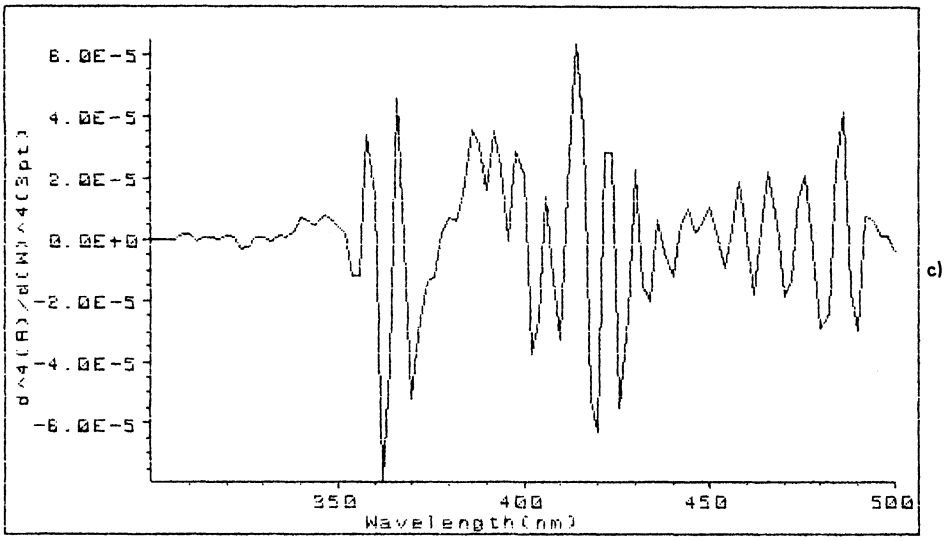
In order to cut down on the necessary storage space, the *sliding arithmetic mean,* or *sliding time average,* is taken for noise elimination (see Section 3.6.4.1). In this case, the arithmetic mean of corresponding points of the first and second scan is taken, then the resulting mean is averaged with corresponding points of the third scan, and so on. Although the deviations of the *sliding* average from the *true* average are mostly small in fundamental spectra, they become larger with an increase in the order of differentiation (cf. Fig. 4-13 and [16]). Therefore, if somehow possible, the true average must be chosen, because the sliding average is only the average of the last mean and the last scan.


0.001
0.000
-0.001
-0.002
d^2(R)/d(W)^2(3pt)
350
400
450
500
Wavelength(nm)

a)


0.001
0.000
-0.001
-0.002
d^2(R)/d(W)^2(3pt)
350
400
450
500
Wavelength(nm)

b)



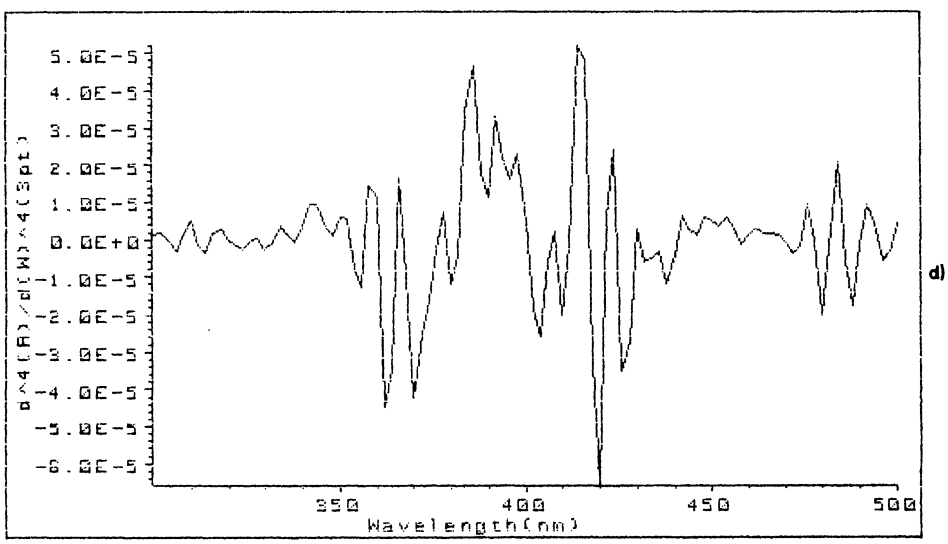


Figure 4-13. Comparison for the true arithmetic mean of 20 independent scans of a $NiCl_2 \cdot 6\ H_2O$ solution in water with the sliding average of 20 scans of the same substance. a) d^2 of the ten averaged fundamental spectrum. b) d^2 of the sliding averaged fundamental spectrum. c) d^4 of the ten averaged fundamental spectrum. d) d^4 of the sliding averaged fundamental spectrum.

4.4.2.3 Response Mode

The response mode is a digital method in which the arriving data is accumulated during a given time interval t (mostly between 0.5 and 20 s), and the smoothed signal is returned with a retardation in time. In this case, the start of the data source must be advanced by the value of the selected response time. Thus the wavelength scan must not be started at λ but at Δ nm *before* the evaluated λ region. Of course, this signal is also more or less distorted relative to those obtained with other smoothing operations. Moreover, the resolution goes down and the signal becomes flatter with rising response time (Fig. 4-14).

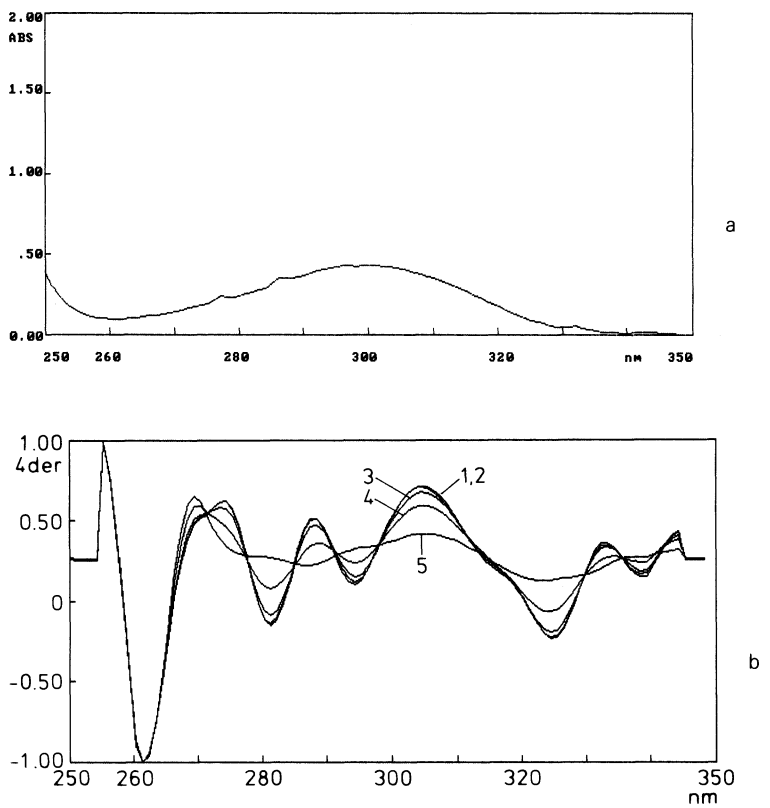


Figure 4-14. Influence of the response time on the fourth derivative of a holmium peak (range: 250–350 nm). Conc. 10 g L^{-1} $Ho(NO_3)_3 \cdot 5\ H_2O$ (slit: 1 nm; scan: 1 nm s^{-1}; data interval: 1 nm).
a) Fundamental spectrum; response: 0.5 s; b) 4th derivative; varying response: 0.5 s (1); 1 s (2); 5 s (3); 10 s (4); 20 s (5).

4.5 Differentiation

In 1978 we developed the first low-noise on-line and analog device for studying derivatives up to the 9th order [1–3, 21]. In the meantime, numerous other applications have been cited in the literature by myself and many other scientists, because the quality of the spectra as well as the possibility of accommodation to a given problem are very high. The results are obtained on-line immediately and are continuously visible. Nevertheless, the immense progress made in semiconductor technology in the last decade and the downward trend of PC prices have led to a shift from analog to digital techniques. But to get the same quality in *higher*-order spectra with digital devices that is obtained using analog equipment, great demands must be made upon the data resolution (16 or 32 bit), memory capacity, and software involved in noise elimination, data manipulation, and differentiation algorithms (see Sec. 3.6.4.2). Otherwise, unsatisfactory results are obtained [2, 16, 18].

4.5.1 Analog Differentiation

The resolution of analog derivative spectra depends not only on the slit width but also to a large extent on the rate of the wavelength scan s and on the differentiation time constant τ. The effect of these three parameters can be seen in Figs. 4-15, 4-16, 4-17, and 4-18.

The spectra in Fig. 4-15 are taken *without* filtering or smoothing. The second derivative contains so much noise already that in this case it is pointless to generate higher-order derivatives.

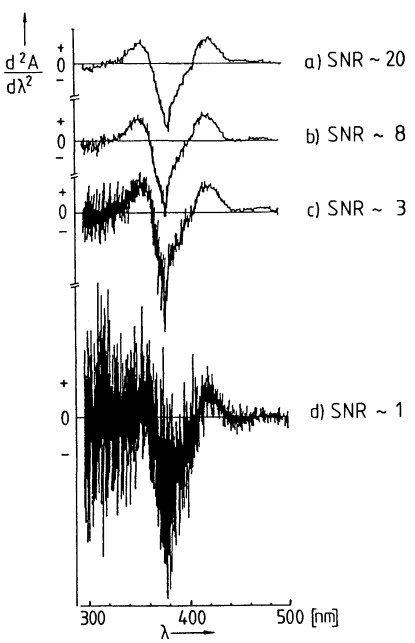


Figure 4-15. Influence of the slit width on the SNR of $NiCl_2 \cdot 6\ H_2O$. Conc. 40 g L^{-1} in H_2O, d^2 without filtering; MD: H.
a) slit: 2.0 nm; b) slit: 1.0 nm; c) slit: 0.5 nm; d) slit: 0.2 nm [14, 15].

A higher scan velocity gives the derivatives a higher amplitude and improves the SNR. This is demonstrated in Fig. 4-16. On the other hand, the increasing velocity creates a greater shift of the signal both in the ground spectrum and, of course, in the derivatives (Table 4-5). Moreover, the resolution of the spectra is lower (Fig. 4-17); only the main signals are nearly the same, if the scan speed is changed from 2 nm s^{-1} to 5 nm s^{-1}.

The influence of the differentiation time constant τ on the peak height can be observed in Fig. 4-18.

The data of Fig. 4-18 are summarized in Table 4-6. As τ increases, a greater effect is observed on the height of the amplitude A than on the noise.

The best results are obtained if a small slit width, a slow scan speed, and a small value of τ are used. In practice, a compromise must be made because a narrow slit

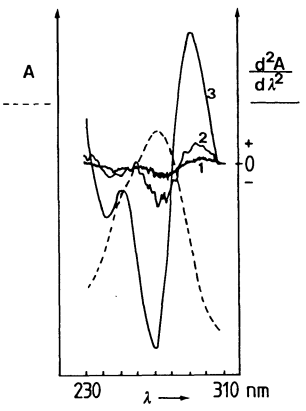


Figure 4-16. Influence of scan velocity S on the SNR of $K_2Cr_2O_7$ (40 g L^{-1} in water). Second derivative for 3 curves (——) (MD: A): curve 1: 1 nm s^{-1}; curve 2: 2 nm s^{-1}; curve 3: 5 nm s^{-1}. (-----) Fundamental spectrum (d^0) [14-15].

Table 4-5. Shift of the peak maximum vs. scan speed[a] fundamental spectra.

scan speed s[nm s^{-1}]	shift Δ [nm]
0.2	0.0
0.5	1.0
1.0	1.0
2.0	2.0
5.0	4.0
10.0	6.0

[a] $NiCl_2 \cdot 6\ H_2O$, conc. 40 g L^{-1} in water.

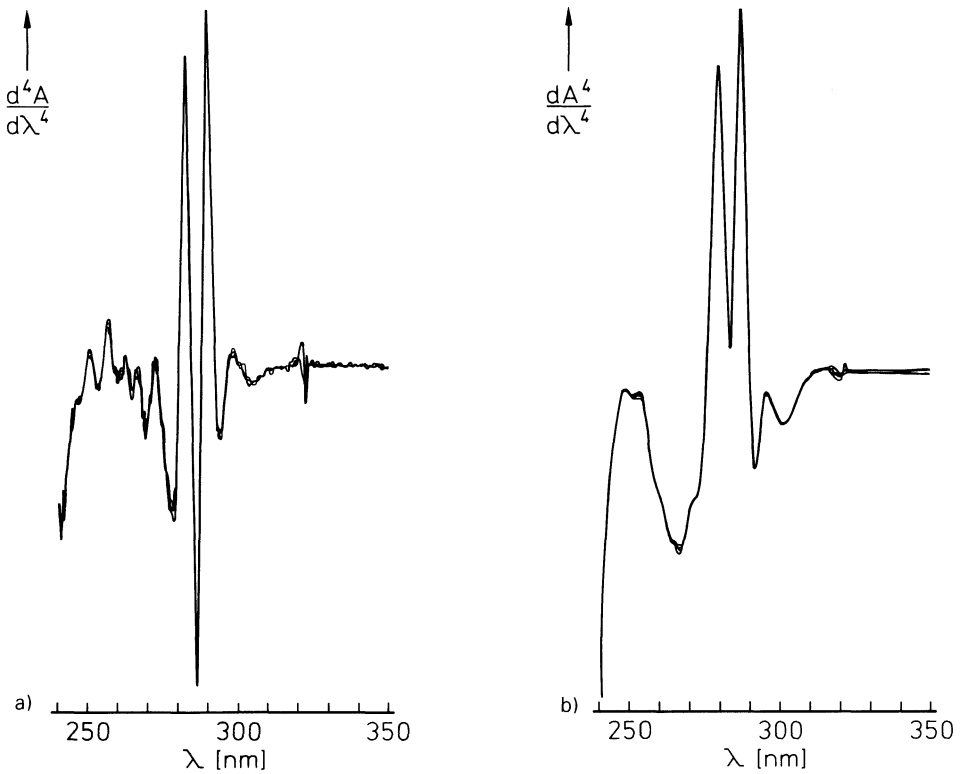


Figure 4-17. Bovine trypsin. Conc. 0.5 g L^{-1}; LTH: 1 cm; slit: 1 nm; d^4; MD: A; sixfold recording;
a) scan: 2 nm s^{-1}; b) scan: 5 nm s^{-1} [2].

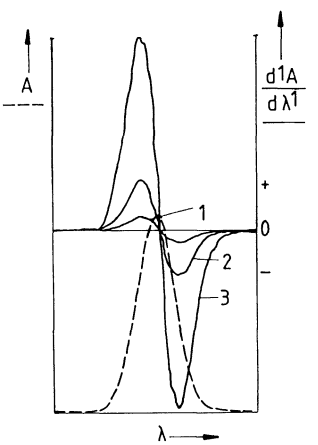


Figure 4-18. Influence of the time constant τ of differentiation on the amplitude of a computed Gaussian peak. First derivative (d^1), MD: A (A = 100 mm, FWHM = 16.65 mm); 1) t = 0.47 ms, 2) τ = 4.7 ms, 3) τ = 9.4 ms (see Table 4-6), (----) fundamental spectrum [14, 15].

Table 4-6. Influence of τ on the amplitude height A of a computed Gaussian peak d^1; MD: A [15].[a)]

Curve	R KΩ	C μF	τ ms	A mm
1	10	0.047	0.47	8.5
2	20	0.470	4.70	31.5
3	30	0.940	9.40	123.5

requires high amplification which leads to increasing noise. If both S and τ are small, only small differences in potential are obtained and therefore the peaks are also small [2]:

$$\frac{dA}{d\lambda} \approx \frac{dA}{dt} \sim \frac{dU}{dt} . \tag{4-2}$$

For the first derivative the time resolution T amounts to

$$T_1 \sim 4\,\tau_1 = 4\,RC\,[\mathrm{s}] \tag{4-3}$$

and for the second derivative

$$T_2 \sim 8\,\tau_2 = 8\,RC\,[\mathrm{s}] \tag{4-4}$$

and for the third

$$T_3 \sim 16\,\tau_3 = 16\,RC\,[\mathrm{s}] \tag{4-5}$$

etc.

This gives the resolution R_D of the differentiator:

$$R_D = TS\,[\mathrm{nm}] \tag{4-6}$$

(S = scan [$\mathrm{nm\,s^{-1}}$]) .

In optimizing the differentiator, the following holds true:

$$R_D \leqq B\,[\mathrm{mm}] \tag{4-7}$$

(where B is the spectral band width of the apparatus) and

$$T < \frac{B}{S}\,[\mathrm{s}] . \tag{4-8}$$

In most cases, the following parameters are advisable:

- spectral band width B 0.2–2 nm
- scan rate S 1–5 $\mathrm{nm\,s^{-1}}$
- differentiation time constant τ 0.5 ms–1 s.

An example may make these considerations more concrete: The data chosen for a spectrophotometer are 1 nm for B and 1 nm s^{-1} for S. For high resolution, e.g., T_1 must be smaller than 1 s, say, 0.5 s. Then R_D is 0.5 nm.

If the capacity of the differentiation circuit is 1 μF (= 10^{-6}F), then for the 1st derivative R must be 125 KΩ, τ_1 125 ms and the $\Delta\lambda_1$ shift 0.5 nm. For the 2nd derivative T_2 becomes 1 s and $\Delta\lambda_2$ shift is then 1 nm. Analogously for D3 and D4 T_3 is 2 s, and T_4, 4 s, while $\Delta\lambda_3$ is 2 nm, and $\Delta\lambda_4$, 4 nm. If the scan is taken from high to low λ, the sign for $\Delta\lambda$ is negative, that is, a shift is made to lower λ data and vice versa. If necessary, the data must be corrected by calibration with a known λ position of a standard. The mean of two scans made in both directions can also be taken.

In order to minimize the stray light, filters must be positioned in the light beam at certain wavelengths. If this is not done continuously, an irregularity results, and a ghost peak appears in the derivative spectrum. A change in the light source in the range of 290 to 340 nm causes a similar interference. This must be kept in mind and, if possible, the switching point has to be shifted or the scan stopped while the filter or lamp are being changed.

4.5.2 Digital Differentiation

Nowadays, a great deal of suitable software for manipulation and differentiation of digital signal are available. If you are not willing to pay $ 2000 or more for the convenience of preprogrammed software (MS-DOS or others), you will have to write your own program (e.g., in BASIC, C, PASCAL, or FORTRAN).

4.5.2.1 Savitzky-Golay Polynomial

The most common algorithm is that of Savitzky and Golay [22, 23]. This polynomial incorporates a shortened least-square computation for data smoothing (see also Section 3.6.4.2). Some points must be considered when using this differentiation mode:

- The absolute values of the computed derivative data are small. Therefore, they must be multiplied by a factor of 100 or more to obtain useful signal heights. Unfortunately, the noise is also multiplied at the same time (Fig. 4-19). In analog differentiators, the amplification is coupled with rising resistance R of the time constant τ.
- At best, the five-point differentiation polynomial is suitable up to the second order. For higher derivatives, an 11- to 25-point polynomial is required.
- If the polynomial has a data set of $(2\ m + 1)$, m computed data at the beginning and m points at the end of the derivatives are artifacts (see Sec. 3.6.4.2).
- Better results are obtained if the signal is differentiated four times by a d^1 polynomial than once by a d^4 polynomial.
- The highest order of the integrated smoothing polynomial is not always the best result. In Fig. 4-20, it is shown that the quadratic-cubic polynomial generates less noise than the quartic-quintic polynomial.

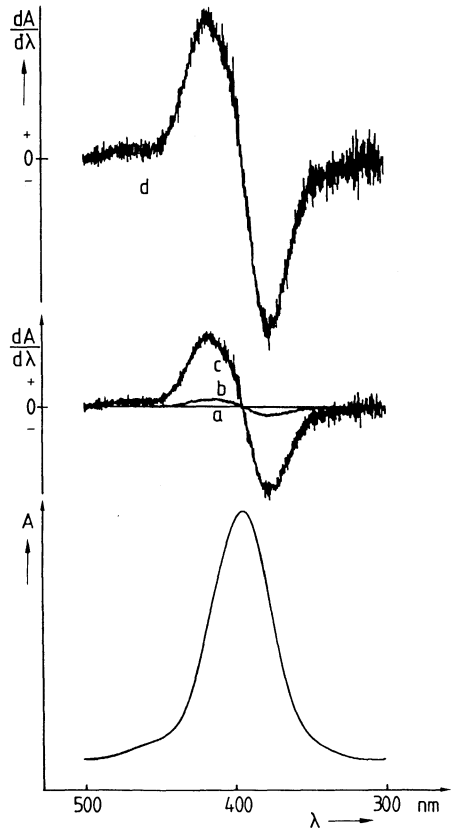


Figure 4-19. Influence of amplification V on the peak height. First derivative of $NiCl_2 \cdot 6\ H_2O$ (40 g L^{-1} in water); MD: D; 5 point S-G; differentiation polynomial (quadratic-cubic).
a) $V = 1$ (practically straight line); b) $V = 5$; c) $V = 50$; d) $V = 100$ [14, 15].

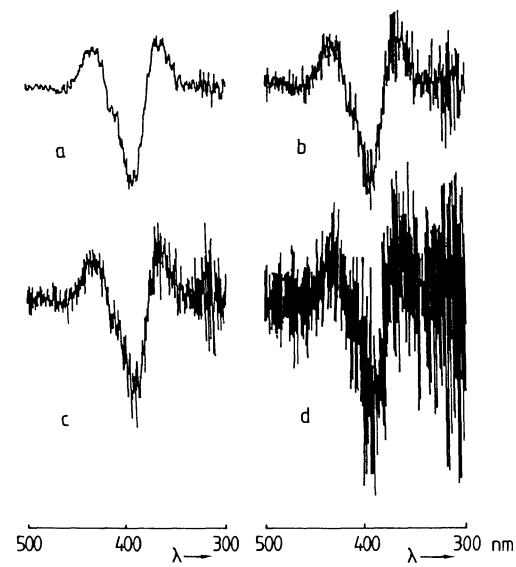


Figure 4-20. Influence of smoothing polynomial order on noise. Second derivative of $NiCl_2 \cdot 6\ H_2O$ (40 g L^{-1} in water); MD: D; (S-G).
a) 25 points, quadratic-cubic; b) 25 points, quartic-quintic; c) 15 points, quadratic-cubic; d) 15 points, quartic-quintic [14, 15].

- The scan speed does not shift the position of the extrema but, if the data points have been time triggered, the increasing scan speed leads to less noise and also to less information. However, if the data points are triggered by the wavelength scale, rising scan speed has no influence when the time difference between two data points is not smaller than usually 10 ms. (This depends on the velocity of the analog-to-digital converter and of the PC.)
- The number of original data points is not diminished by increasing the point width of the polynomial. If the PP difference quotient method is used, though, the number of points is diminished.

4.5.2.2 Point-Point Differentiation

Another differentiation method is point-point differentiation (see Sec. 3.6.4.2), also called the difference quotient method. We obtained very good results with this technique by following simple mathematical manipulations:

a) Elimination of noise by accumulation of independently taken spectra (or other signals) and computation of the true arithmetic mean. In this case, no deformation or distortion of the curves takes place. In our opinion this is the best way to eliminate noise (Fig. 4-21). The accumulation of 10 scans leads to an improvement of the SNR by a factor of two in the original curve and by a factor of four in the second derivative. If it is not possible to take more than one or two scans, the 3- or 5-point sliding average (sliding window) is also practicable (Sec. 3.6.4.1). If necessary, this operation must be repeated five to ten times. It is more favorable, for example, to take a 3- or

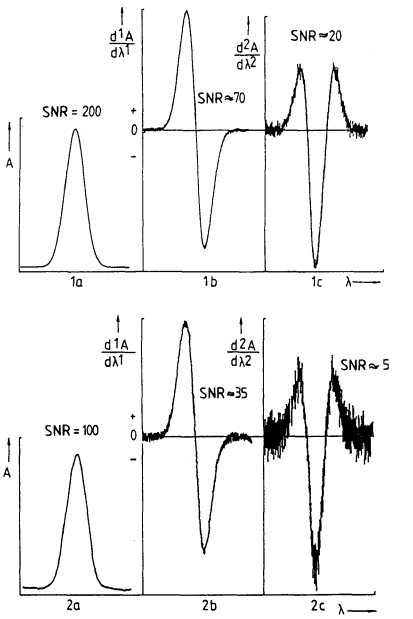


Figure 4-21. Improvement of the SNR by accumulation and averaging of electric signals. Synthetic Gaussian band (A = 100 mm, FWHM = 16.65 mm): 1a–1c: d^0, d^1, d^2; Tenfold scan averaged. 2a–2c: d^0, d^1, d^2; not averaged. MD: D (PP).

5-point sliding window twice than it is to take a 6- or 10-point set of data only once. It must be kept in mind that *all* smoothing operations involve a compromise between a reduction of the noise with an alteration of the shape of the signal and the loss of information.

b) The next step is the computation of the difference quotient (see Sec. 3.6.4.2). The influence of the differentiation width $\Delta x \triangleq h \triangleq \Delta\lambda$ is clearly seen in Fig. 4-22. A higher SNR (lower noise) corresponds to a smaller signal height, but also to lower resolution. The SNR rises very strongly with Δx (Fig. 4-23 a). If Δx is 1 nm, the SNR goes up from 10 to about 100. On the other hand, the amplitude of the signal goes down only modestly if Δx increases (Fig. 4-23 b). It must be noted that for generating higher-order derivatives it is often better not to take the same Δx for all orders (e.g., for d^4 1.5, 1.5, 1.5, 1.5), but to raise the Δx values (e.g., 0.5, 1, 1.5, 2).
For computing the difference quotient $\Delta y/\Delta x$, the difference of two amplitude values is divided by Δx , the width of differentiation and the differentiated value mostly positioned in the middle of Δx, say at $\Delta x/2$. For this reason, the data of the derivatives and, therefore, also the positions of the extrema are shifted for $\Delta x/2$ in the direction of differentiation. This alteration of the beginning of the curve can be exactly predetermined and therefore easily compensated for.

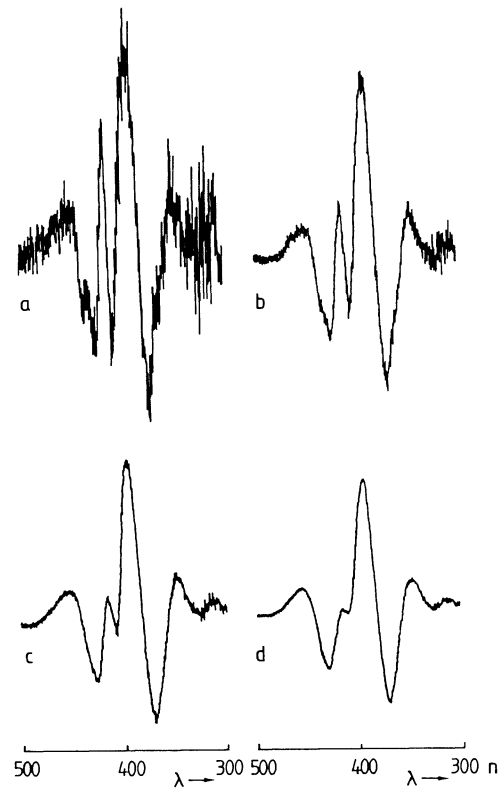


Figure 4-22. d^4 of $NiCl_2 \cdot 6\ H_2O$ (40 g L^{-1} in water). Fourfold differentiation by Δx (difference quotient mode); MD: D (PP).
a) $\Delta x = 3$ mm; b) $\Delta x = 4.0$ mm;
c) $\Delta x = 5.0$ mm; d) $\Delta x = 6.0$ mm [15]
(1 mm ≙ 1 nm).

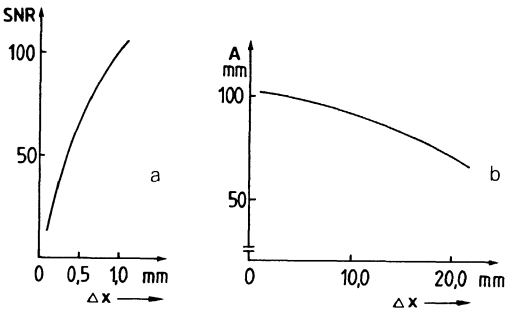


Figure 4-23. Influence of Δx ($NiCl_2 \cdot 6\ H_2O$, 40 g L^{-1} in water); first derivative; MD: D (PP).
a) Alteration of SNR vs. Δx; b) Alteration of the amplitude of the signal vs. Δx [15] (1 mm ≙ 1 nm).

In Table 3-9 some more complex algorithms are listed. One of them, the Fourier series, has been used more frequently in the past few years. The reason for this may be the great progress in the hard- and software for digital computation. Nevertheless, one should first evaluate in each case whether it is really necessary to generate derivatives by more complicated algorithms, and whether the quality and resolution of the spectra can be improved in this way.

4.5.3 Quality of Derivatives

In order to compare the quality of derivatives, taken under different conditions by different methods, or if parameters for the generation of derivatives have to be optimized, qualitative criteria, or preferably quantitative criteria, need to be available. Of course, everyone prefers spectra with the lowest amount of noise and the highest resolution. Once again, this is not feasible. A compromise must always be made between these two extremes. Yet with careful and thorough work, analog or digital modes give very good results. In Fig. 4-24 a tenfold d^4 scan of Congo Red is taken by an analog differentiator,

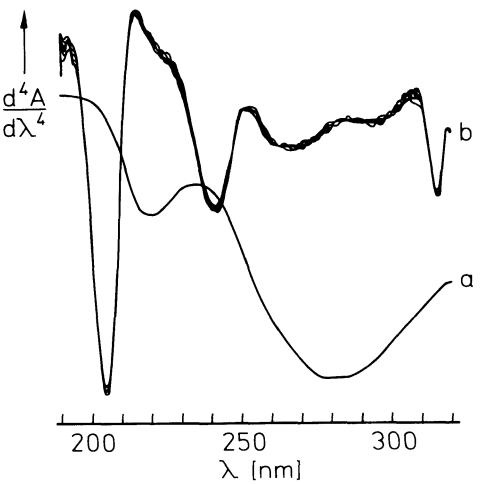


Figure 4-24. Reproducibility of spectra with Congo Red as an example. Conc. 20 mg L^{-1} (LTH: 1 cm; slit: 1 nm; scan: 5 nm s^{-1}; d^4; MD: A; overlay tenfold) [2].
a) Fundamental spectrum; b) fourth derivative; overlay tenfold.

and in Fig. 4-25 a threefold d^2, d^4, and d^6 scan of $NiCl_2 \cdot 6\ H_2O$ is given. In both cases the reproducibility is very good.

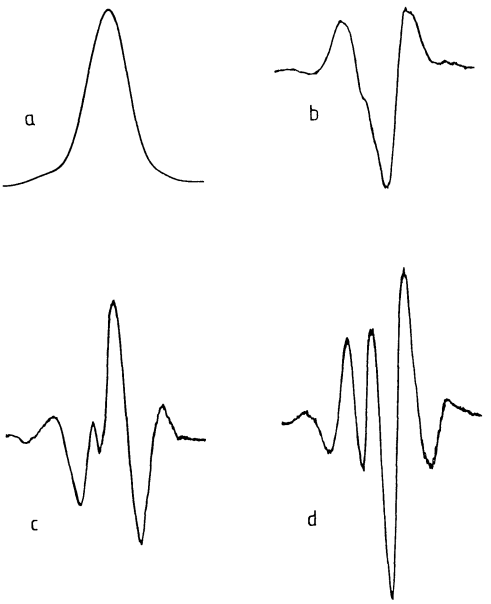


Figure 4-25. Reproducibility of spectra. $NiCl_2 \cdot 6\ H_2O$. Conc. 40 g L^{-1} (threefold overlay).
a) d^0; b) d^2; c) d^4; d) d^6. MD: D (PP) corrected [15].

4.5.3.1 Noise Estimation

The data of individual measurements fluctuate irregularly around an effective value (random errors). These deviations are caused by the noise of the signal source. The absolute value of the noise, which depends on different experimental conditions, can be estimated from the deviations of the base line. In spectroscopy it is also possible to use the spectra of optical attenuators, e.g., of a sieve or a grey filter. Then the noise must be minimized by optimizing the on-line low-pass output filter of the apparatus or the gate filter of the derivative device. The dimension of the noise is given in [mm].

4.5.3.2 Signal-to-Noise Ratio

Numerous independent scans of a substance under the same conditions give numerous peaks with an average height S. The difference between the highest and lowest peak height is the noise N (Fig. 4-26).

Having a constant amplification, the noise is independent of the signal height of two different peaks and, consequently, the SNR is directly proportional to the peak height according to

$$\frac{S_1}{S_2} = \frac{\mathrm{SNR}_1}{\mathrm{SNR}_2} . \tag{4-11}$$

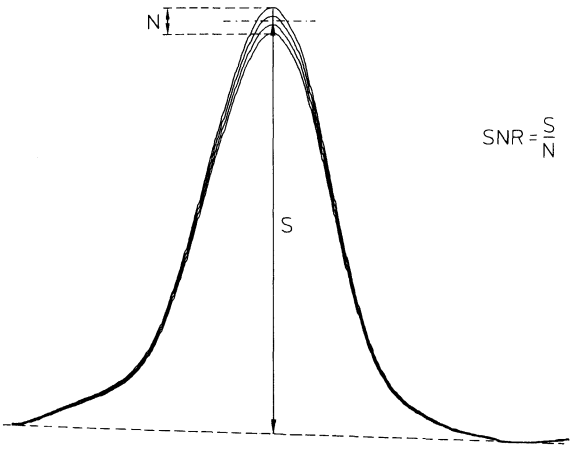


Figure 4-26. Computation of SNR (qualitative graph). For example, if the mean peak height is 100 mm and the noise level 5 mm, then the SNR becomes 20 and the highest deviation of the peak height is ±2.5%.

If only a single scan is possible, the noise must be estimated, as shown in Sec. 4.5.3.1. If many spectra are available, it is better to take the arithmetic mean of the maxima. The plus-minus mean-error deviation is then a useful value for characterizing the noise.

4.5.3.3 Signal-to-Signal Ratio

It is not only important to know the reliability of the data but also to know how well or how insufficiently superposed signals are resolved. Information is given by comparing the values of the *signal-to-signal* ratio (*SSR*), which can be easily computed from the ratio of a main peak and its shoulder or inflection point at its flank:

$$\mathrm{SSR} = \frac{S_1}{S_2} = \frac{\text{shoulder height}}{\text{main peak height}} .$$

In Fig. 4-27 the SSR of the fourth derivative is 0.12 and that of the 6th derivative, 0.50; this means that the resolution is essentially better for the higher derivative.

The dependence of the SNR and SSR on the differentiation width for the d^4 of $NiCl_2 \cdot 6\ H_2O$ in Fig. 4-22 is given in Fig. 4-28. While the SNR rises rapidly with increasing Δx, the SSR goes down rapidly.

Independent of the question of whether high resolution or low noise is desired, a Δx must be chosen which gives the highest degree of information.

The influence of the smoothing width on the resolution of two computed Gaussian functions with superposed noise and a different smoothing ratio r, that is, the ratio of the smoothing width to the FWHM

$$r = \frac{\text{number of smoothing points}}{\text{FWHM}} \qquad (4\text{-}11)$$

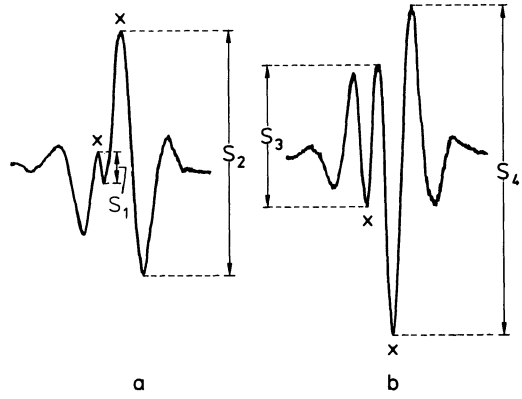


Figure 4-27. SSR computation.
$NiCl_2 \cdot 6\ H_2O$. MD: D (PP).
a) d^4 (original maxima are positive);
b) d^6 (original maxima are negative).

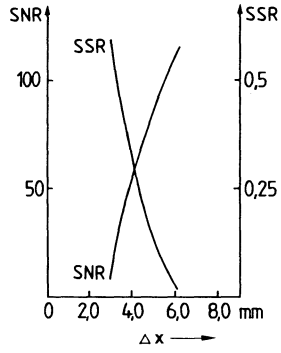


Figure 4-28. Influence of Δx on SNR and SSR of the fourth derivative of $NiCl_2 \cdot 6\ H_2O$ (see also Fig. 4-22); MD: D (PP) [15].

was also tested, where A_1: 100 mm; A_2: 50 mm; $FWHM_1$: 27,78 mm; $FWHM_2$: 27,78 mm; and *t:* 15.0 mm. Especially when r is high ($r = 0.90$), the resolution goes down. In the first derivative, the best results are obtained by smoothing with a 15-point polynomial quadratic-cubic (according to the Savitzky-Golay method) – the result is a high SNR with high resolution. On the other hand, a 5-point polynomial is considerably noisy, and the 25-point polynomial is not very well resolved. In addition, in the second derivative the 15-point polynomial gives the best results. The other d^2 curves either display background noise or have lower signals (Fig. 4-29).

A comparison of a computed Gaussian peak with the weak asymmetric spectrum of $NiCl_2 \cdot 6\ H_2O$ demonstrates the high resolution power of the derivative technique and indicates that the asymmetry of the latter is caused by a second superposed signal. In the second-derivative spectrum of $NiCl_2 \cdot 6\ H_2O$, an inflection point is already noticable. This splitting rises gradually to a second peak with an SSR of 0.11 in the 4th order and 0.47 in the sixth order. This proves that the peak splitting is not an artifact but actually the fine structure of this substance (Fig. 4-30).

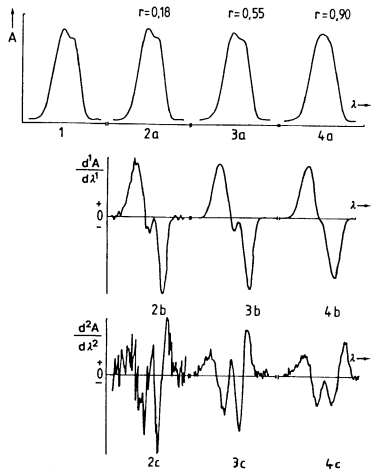


Figure 4-29. Influence of the smoothing width on the resolving of two superposed Gaussian peaks with background noise; MD: D (PP). Smoothed according to Savitzky-Golay method [15].
1: d^0 (A_1 100 mm, A_2 50 mm, $FWHM_1$ and $FWHM_2$ 27.78 mm) unsmoothed; 2a-2c: d^0 (5 point smoothing), d^1, d^2; 3a-3c: d^0 (15 point smoothing), d^1, d^2; 4a-4c: d^0 (25 point smoothing), d^1, d^2.

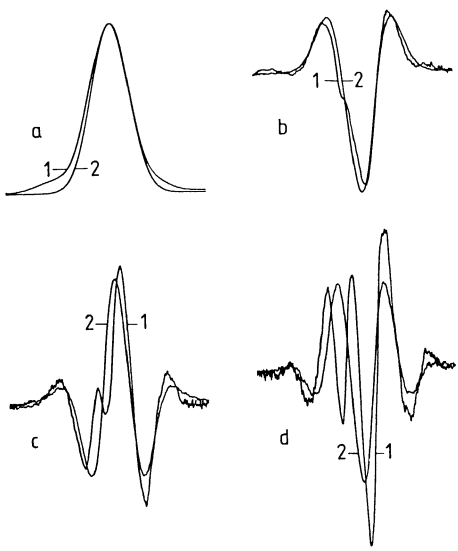


Figure 4-30. Comparison of the spectrum of $NiCl_2 \cdot 6\,H_2O$ (curve 1) and a computed Gaussian peak (curve 2); A = 84,0 mm, FWHM = 21.81 mm.
a) d^0; b) d^2; c) d^4; d) d^6; MD: D (PP) [15].

4.6 Evaluation of Derivatives

In Sec. 2.6 the possibilities for evaluation of derivatives were described in detail. Now some special evaluation techniques may be treated and demonstrated with practical examples.

First, we must pose the question of whether an odd or an even order shall be evaluated. In practice, mostly even orders are used because the peaks of the derivatives

correlate with the peaks and shoulders of the fundamental spectra. In a number of cases, if the even derivative spectra do not lead to satisfying results, odd derivatives just may provide the solution to the problem. Evaluation of odd orders often gives better results for resolving absorption edges or inflection points (Fig. 4-31) or for analysis of two or more components (e.g., [2, 25–28]).

For quantitative measurement, all methods are suited which evaluate the signal *directly,* such as the peak-peak, peak-tangent, and peak-zero methods (Sec. 2.6.1.1–2.6.1.3). However, if derivatives need to be compared, the signals must be normalized, which means that the concentration must be identical or it must be eliminated.

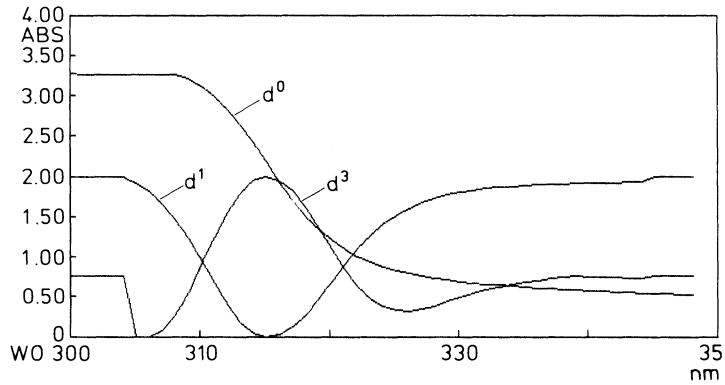


Figure 4-31. Odd derivatives of a spectrum of a plate of $LiNbO_3$ with a thickness of 1.76 mm (d^0, d^1, d^3) (slit: 1.0 nm; scan speed: 1.0 nm s^{-1}; data interval: 1.0 nm; response: 5 s); MD: D (S-G 11). The extrema of d^1 and d^3 coincide with the inflection point of d^0.

4.6.1 Normalization by Multiplication or Division

It is possible to amplify the highest peaks in both analog spectra or to multiply the digital data by a factor that allows at least two signals to be congruent. Then the possible deviations can be observed on the monitor or after plotting them out.

Another method is to take the ratios of the peak heights of both spectra and to compare the results (Sec. 2.6.1.4). The ratios must be equal in the case of identical substances even if the concentrations are different (PPR method). This mathematical operation can be carried out by a short computer program. The printout is a set of data given in Table 4-7 for the comparison of trypsin and acetyltrypsin. Note that even small differences in the substances can easily be detected. In the fundamental spectra, the shape of the curves are very similar (Fig. 4-32a) but the differences become clearly visible if the heights of the corresponding peaks in Fig. 4-32b are mathematically manipulated. Other examples, such as the comparison of different varieties of beer [2], whale and horse myoglobin [25], and human and bovine hemoglobin [5] can be found in the cited literature.

Table 4-7. PPR computation of d^4 spectra of trypsin and acetyltrypsin. (The closer the data of the ratio PPR_1/PPR_2 are to 1.0, the more similar the compared peaks will be.)

Peak	Trypsin Peak Height mm	PPR_1	Acetyl-Trypsin Peak Height mm	PPR_2	%[1]	$\frac{PPR_2}{PPR_1}$
AB	84.0		75			
		3.65		3.95	+ 8.2	0.92
CD	23.0		19.0			
		2.05		1.58	22.9	1.30
FG	11.2		12.0			
		0.68		0.92	+35.3	0.74
HI	16.5		13.0			
		0.21		0.24	+14.3	0.88
JK	77.4		55.0			
		0.35		0.31	+11.4	1.13
LM	222.0		178.5			
		1.60		1.28	−20.0	1.25
NO	139.0		140.0			
		5.11		5.60	+ 9.6	0.91
PQ	27.2		25.0			

[1] Percent deviation of PPR_2 from PPR_1.

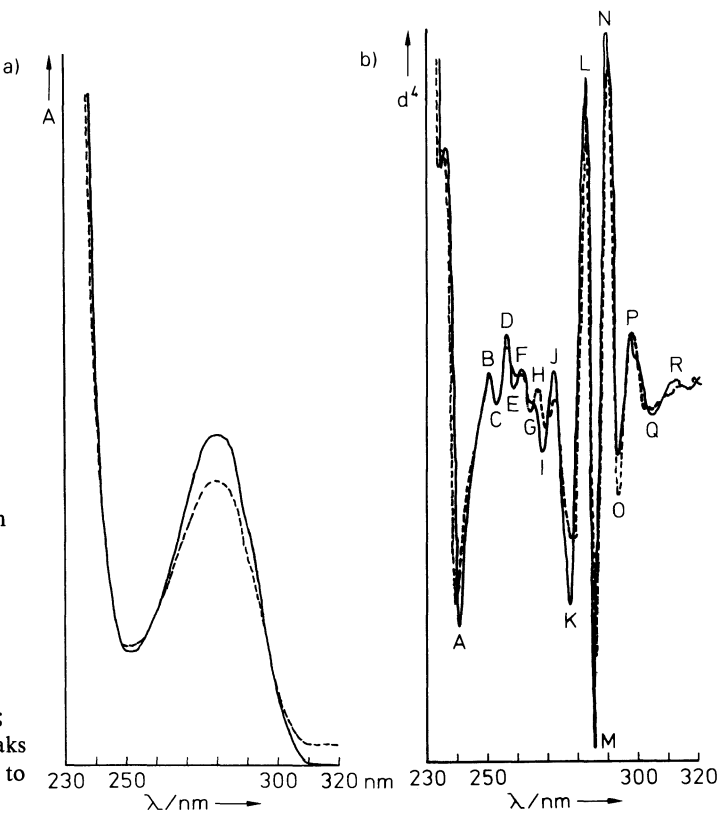


Figure 4-32. Peak-peak ratio evaluation. Trypsin conc.: 0.5 g L^{-1} (——); Acetyltrypsin conc.: 0.5 g L^{-1} (- - - -) (slit: 1.0 nm; scan speed: 2 nm s^{-1}; LTH: 1 cm); MD: A.
a) Fundamental spectra; b) fourth derivative (peaks marked by the letters A to R).

4.6.2 Partitive HODS

In order to compare two spectra visually, all points of a curve A are divided by the corresponding points of the curve B at the same wavelength region (partitive method, see Sec. 2.6.3.3). This has been carried out in Fig. 4-33. The spectra are taken from different concentrations of chymotrypsin. Theoretically, the division of both curves leads to a straight line, because this manipulation normalizes the data. In practice, small deviations caused by residual noise may occur [10, 29] (see Fig. 4-33, 1c to 4c).

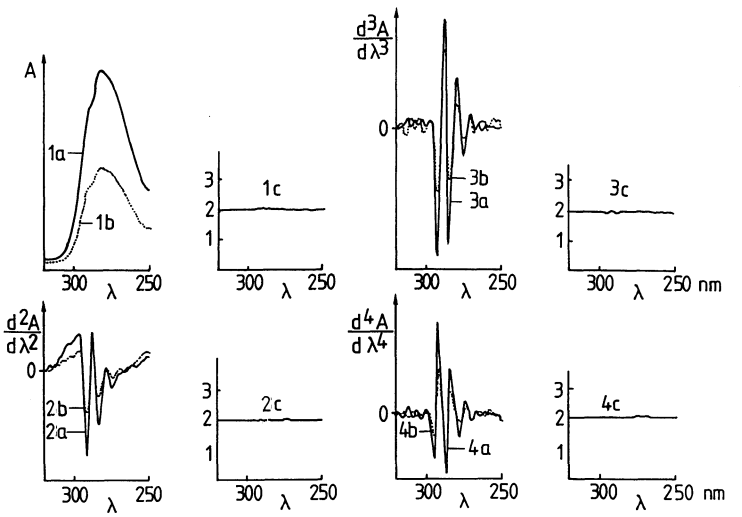


Figure 4-33. Partitive HODS. 1a: Fundamental spectrum of chymotrypsin; conc. 0.250 g L^{-1} (——); 1b: fundamental spectrum of chymotrypsin, conc. 0.125 g L^{-1} (-----); 2a, 2b: d^2 of 1a 1a and 1b; 3a, 3b: d^3 of 1a and 1b; 4a, 4b: d^4 of 1a and 1b; 1c: division of 1a: 1b; 2c: division of 2a: 2b; 3c: division of 3a: 3b; 4c: division of 4a: 4b [29, 30]; MD: D (PP) [10, 29].

Comparison of spectra of *different* substances by partitive HODS does not result in a straight line, because the ε values are not identical, and therefore the conditions of Eqs. (2-74)-(2-76) are not fulfilled. Moreover, it must be noted that the heights were always taken from zero to the positive or negative value of the curves and not from a positive quantity to the lowest negative quantity of the signal or to any base line (bipolar division).

4.6.3 Side-Peak-Side Ratio Method

The side-peak-side ratio method is a special variant of the PPR method and a very effective evaluation technique if similar spectra need to be compared (Sec 2.6.2.4). Principially, one can take the ratios of the sides of only *one* single peak or of a number of maxima and/or minima, or preferably that of *all* extrema. The normalized and tabulated

data show numerically which parts of the spectra are equal or similar and which parts are different. In this way whale and horse myoglobin was characterized [25] as well as human and bovine hemoglobin [5].

A very clear graphical illustration of the SPSR data can be created (see Sec. 2.6.2.4 and [30, 31]). In Fig. 4-34b, fourth derivatives of pigment green 7 (PG 7) and pigment green 36 (PG 36) are given. Their chemical structures differ from each other only in the substituents of the porphyrine rings of the copper-phthalocyanines. PG 7 has 14 chlorine atoms, PG 36 only 8 chlorine atoms and, in place of the other chloro atoms, 6 bromine substituents. In the fundamental spectrum (Fig. 4-34a), no characteristic difference in the shape can be noted. After SPS ratio computation (Table 4-8), and transferral of the values onto a line drawing (Fig. 4-34c), it is obvious that both pigments have similar spectra, but that deviations occur, particularly at 346, 495, and 580 nm.

It must first be taken into consideration that the ratios are always taken in the same direction, e. g., left-peak height to right-peak height, or vice versa, and second, that all maxima are positive and all minima negative in the line drawing (Fig. 4-34c).

In this connection, the three ratios PPR, SPSR, and SSR are set against each other in Fig. 4-35.

Table 4-8. Computation of SPS ratios of d^4 spectra of PG 7 and PG 36 [32].

PG 7				PG 36			
λ [nm]	S_r	S_l	R	λ [nm]	S_r	S_l	R
724	2.2	3.4	+0.65	726	2.5	3.5	+0.71
705	3.4	1.85	−1.84	705	3.5	1.8	−1.94
686	1.85	0.9	+2.06	686	1.8	0.9	+2.0
674	0.9	2.2	−0.41	674	0.9	3.2	−0.28
652	2.2	2.9	+0.76	654	3.2	4.4	+0.73
634	2.9	3.5	−0.83	634	4.4	5.6	−0.78
614	3.5	2.4	+1.46	616	5.6	6.0	+0.93
600	2.5	1.0	−2.5	600	6.0	2.9	−2.07
592	1.0	3.1	+0.32	588	2.9	2.4	+1.21
583	3.1	2.4	−1.29	583	3.1	2.4	−1.29
495	0.9	0.9	−1.0	470	3.1	5.5	−0.56
480	0.9	1.7	+0.53	455	5.5	3.7	+1.49
465	1.7	2.5	−0.68	440	3.7	2.2	−1.68
450	2.5	1.8	+1.39	425	2.2	2.4	+0.92
433	1.8	1.1	−1.64				
425	1.1	0.8	+1.37				
364	2.8	2.0	+1.4	368	2.6	1.8	+1.44
344	2.0	1.2	−1.67	335	4.2	10.8	−0.39
335	1.2	5.5	+0.22	320	10.8	11.9	+0.91
325	5.5	12.5	−0.44	306	11.9	10.0	−1.19
312	12.5	15.7	+0.80	289	10.0	117.4	+0.57
299	15.7	13.6	−1.15				
288	13.6	18.5	+0.73				

[a] S_r: height of the right peak side; S_l height of the left peak side; R: S_r/S_l

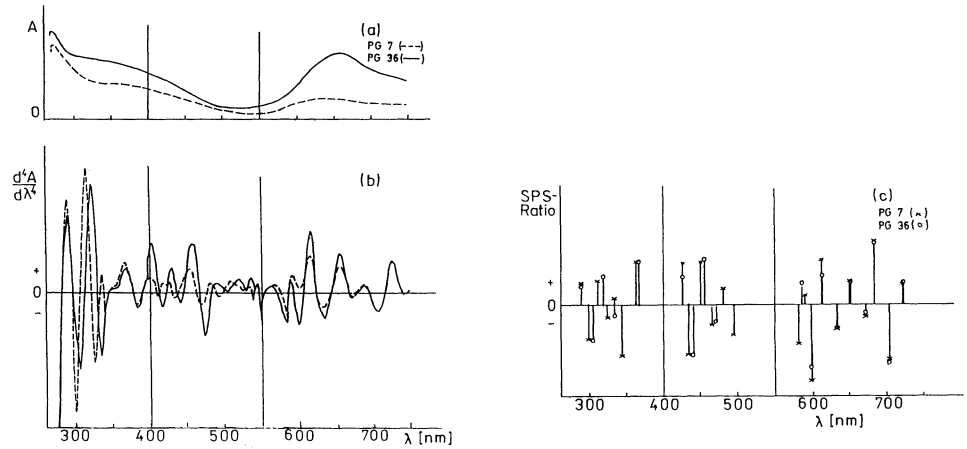


Figure 4-34. Side-peak-side ratio. Comparison of PG 7 (---) and PG 36 (—).
a) Fundamental spectra; b) fourth derivatives of (a); c) SPS ratio line diagram; MD: A (H) [32].

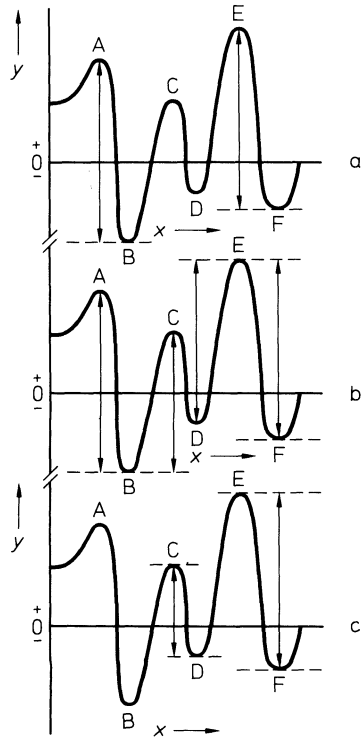


Figure 4-35. Evaluation of derivative spectra.
a) Peak-peak ratio; b) side-peak-side ratio (the ratio of the minimum peak B is negative, the ratio of the peak maximum E positive); c) side-side ratio (gives a measure for resolution of superposed peaks) (schematic drawings).

4.6.4 Additive and Subtractive HODS

It may be of interest to determine whether or not there is a physical or chemical interaction between two substances. This question can be answered by additive HODS, i.e., by comparing the derivatives of a mixture with the sum of the derivatives of the pure substances (Fig. 4-36). (Sec. 2.6.3.1) If they are congruent, no interaction takes place, and vice versa.

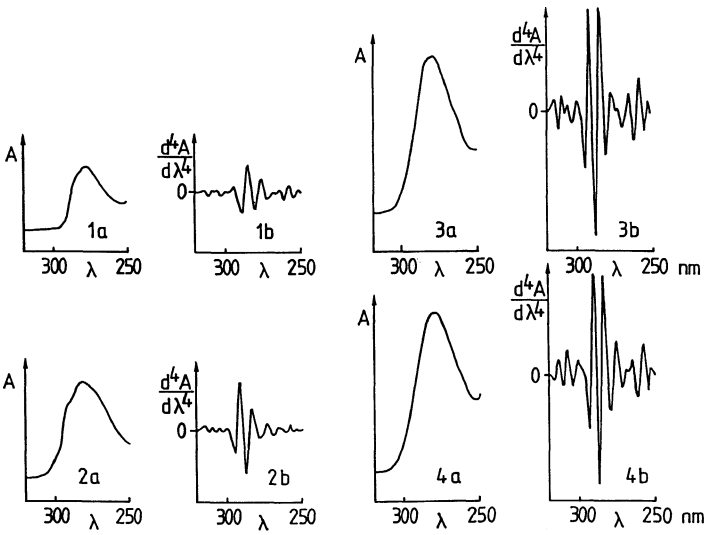


Figure 4-36. Additive HODS. 1a: fundamental spectrum of RNase (conc.: 0.250 g L^{-1} in water); 1b: fourth derivative of 1a; 2a: fundamental spectrum of chymotrypsin (conc. 0.125 g L^{-1} in water); 2b: fourth derivative of 2a; 3a: computed sum of spectra 1a and 2a; 3b: computed sum of derivatives 1b and 2b; 4a: mixture of RNase and chymotrypsin (conc. 0.375 g L^{-1} enzymes); 4b: fourth derivative of 4a. The d^4 spectra 3b and 4b are nearly identical; this means that the substances show only a small, but noticeable interaction; MD: D (PP) [10, 29].

Subtractive HODS enables one to determine an unknown substance from the derivative of a mixture. For example, in a mixture of two components only one of the substances and its concentration is known. Its derivative must then be subtracted from the derivative of the mixture. The remaining derivative belongs to the unknown substance which has to be identified by comparing it with standard spectra in a catalog generated under the same conditions (Fig. 4-37).

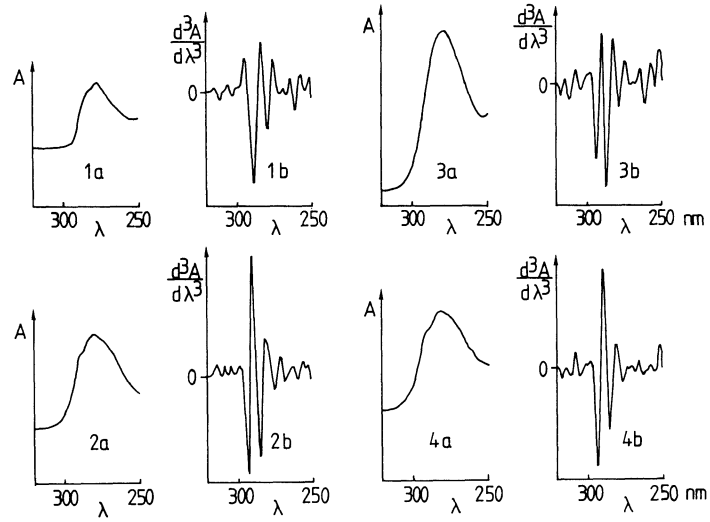


Figure 4-37. Subtractive HODS. 1a: fundamental spectrum of RNase (conc. 0.250 g L^{-1} in water); 1b: third derivative of 1a; 2a: fundamental spectrum of chymotrypsin (conc. 0.125 g L^{-1} in water); 2b: third derivative of 1a; 3a: mixture of RNase and chymotrypsin (conc. 0.375 g L^{-1} enzymes); 3b: third derivative of 3a; 4a: computed subtraction of 3a−1a→4a (almost identical with 2a); 4b: computed subtraction of 3b−1b→4b. The d^3 spectra 2b and 4b show only small differences; MD: D (PP) [10, 29].

4.6.5 Log-A Method

In Sec. 2.6.4.1 we were able to show that the logarithm of different concentrations of the same substance leads to congruent spectra which are only shifted along the *y*-axis. Their shape is undependent of the concentration [10, 29]. If they are differentiated, they are superposed upon each other and no differences should be observed (Fig. 4-38). This type of derivative is used especially for comparing and identifying substances, for standardization, and for creating spectra catalogs and data banks [33].

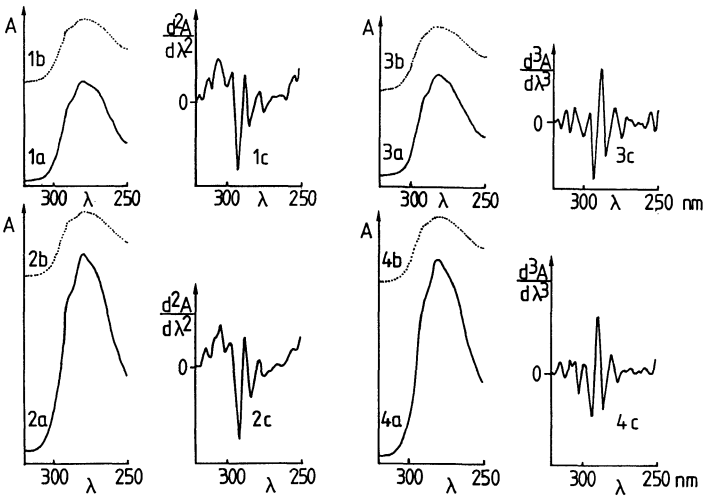


Figure 4-38. Log-A method. 1a: fundamental spectrum of chymotrypsin (conc. 0.125 g L^{-1} in water); 1b: log of 1a; 1c: second derivative of 1b; 2a: fundamental spectrum of chymotrypsin (conc. 0.250 g L^{-1} in water); 2b: log of 2a; 2c: second derivative of 2b. 1c and 2c are practically identical. 3a and 3b resemble 1a and 1b. 3c: third derivative of 3b. 4a and 4b resemble 2a and 2b. 4c: third derivative of 4b. 3c and 4c are also nearly equal and are independent of concentration and order of differentiation. MD: D (PP) [10, 29].

4.6.6 Differentiation–Integration Method

It may be that a spectrum of a substance is only known superposed by some background, e.g., background caused by light scattering in a turbid medium or other circumstances. In this case, it is recommended to take a number of derivatives until the background is eliminated. Then, the reverse function of differentiation, integration, must be carried out to restore the undisturbed fundamental spectrum (Sec. 2.6.4.2, [15, 19], and Fig. 4-39).

Similar to the case of computing derivatives, the peak amplitude depends on the integration width. This effect was studied on a real spectrum which was initially differentiated with $\Delta x = 0.1$ mm. Up to an integration width of $d < 2$ mm, the reduction of the peak height is less than 1%; only if d becomes more than 10 mm the peak is diminished by more than 5% (Fig. 4-40).

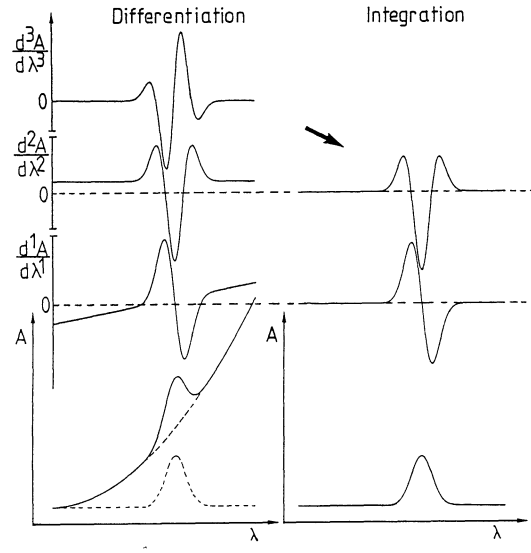


Figure 4-39. Differentiation (d^1, d^2, d^3) of a computed Gaussian band superposed by background (2nd order) and its integration stepwise (integrated twice; data interval: 0.1 mm); MD: D (PP), Δx: 0.1 mm) (all spectra computed) [15, 29].

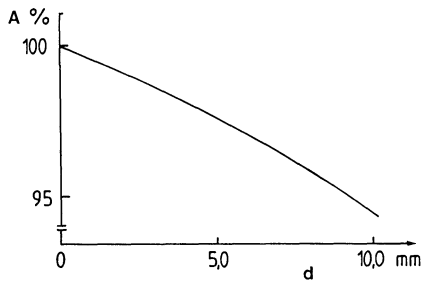


Figure 4-40. Influence of the integration width d on the amplitude of the peak A after integrating the first derivative; MD: D (PP); $\Delta x = 0.1$ mm. Spectrum of $NiCl_2 \cdot 6\ H_2O$ (conc. 40 g L^{-1} in water) [15].

4.6.7 What is the Optimal Derivative Order?

This fundamental question can only be answered generally: the best order is apparent when all shoulders and inflection points are resolved in extrema, oscillating more or less around the baseline. As a rule, this point is reached using the fourth to sixth-order derivative.

Each spectrum, and in general each curve, has an optimal order. Below this optimum amount of information, the resolution is not sufficient, and above this point, the influence of satellites is too great, the derivatives too complicated, or no further information can be obtained, because of the nature of the function; e.g., if the peaks converge to a sine wave, its derivative is a cosine, and a cosine gives a sine by differentiation, and so on. Therefore, only in special cases are higher orders than d^6 used, e.g., to resolve strongly overlapping signals or as fingerprints for identification and comparing of unknown signals. Three examples support this statement.

In Fig. 4-41, the first to ninth derivative was generated from the flat fundamental spectrum of potassium nitrate. We see in this case that d^2 does not resolve the curve. Even d^4 has a shoulder which is only resolved in the sixth to eight order.

In the second example (Fig. 4-42), the fundamental curve of potassium permanganate was differentiated and already shows distinct shoulders in d^0. In this case, the most favorable result is obtained with the second derivative. Higher orders give no more information. The shape of the signal remains practically unchanged. The sine-cosine effect is in this example particularly evident. Finally, if the basic signals are relatively flat and have some small shoulders, as in the spectrum of ribonuclease (or other proteins), the optimal order is in most cases the fourth derivative. It may sometimes be a benefit to take the sixth-order derivative (or an even higher-order derivative) to obtain a fingerprint of the substance (Fig. 4-43).

Other examples can be found in references [2-5, 7, 16].

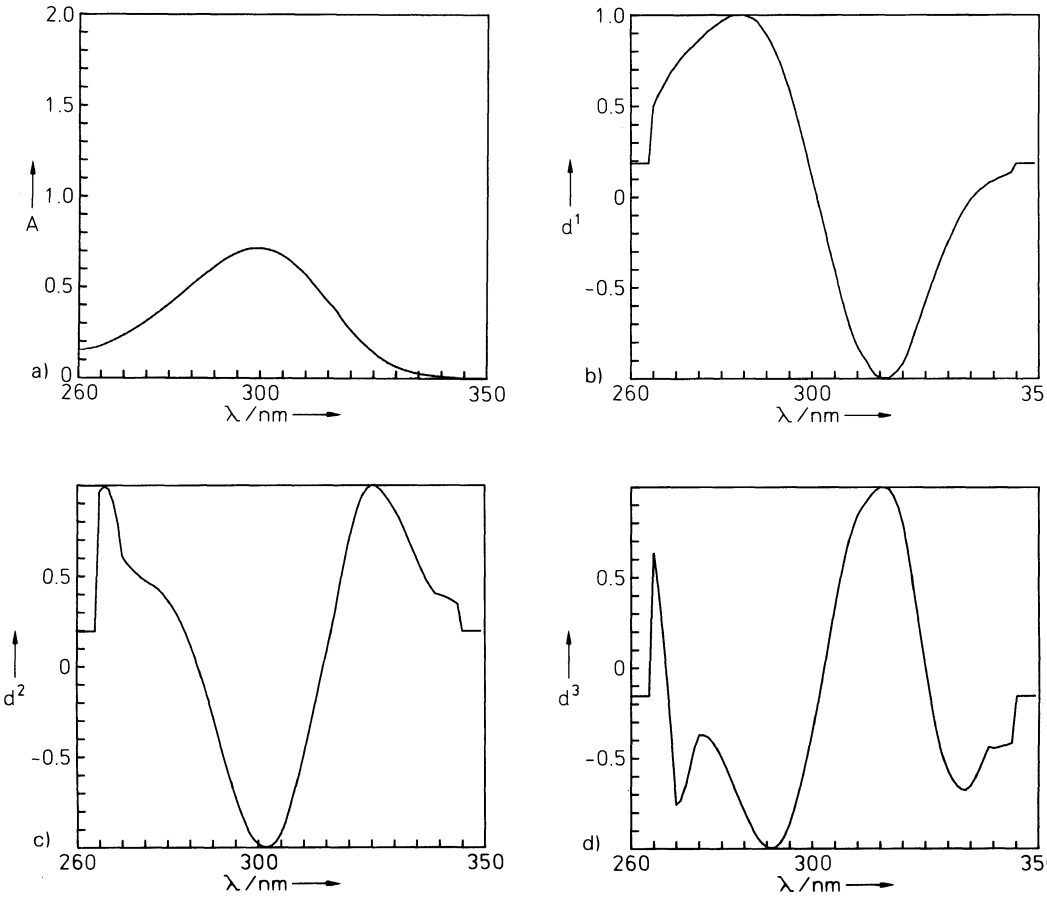


Figure 4-41. Potassium nitrate. Caption next page.

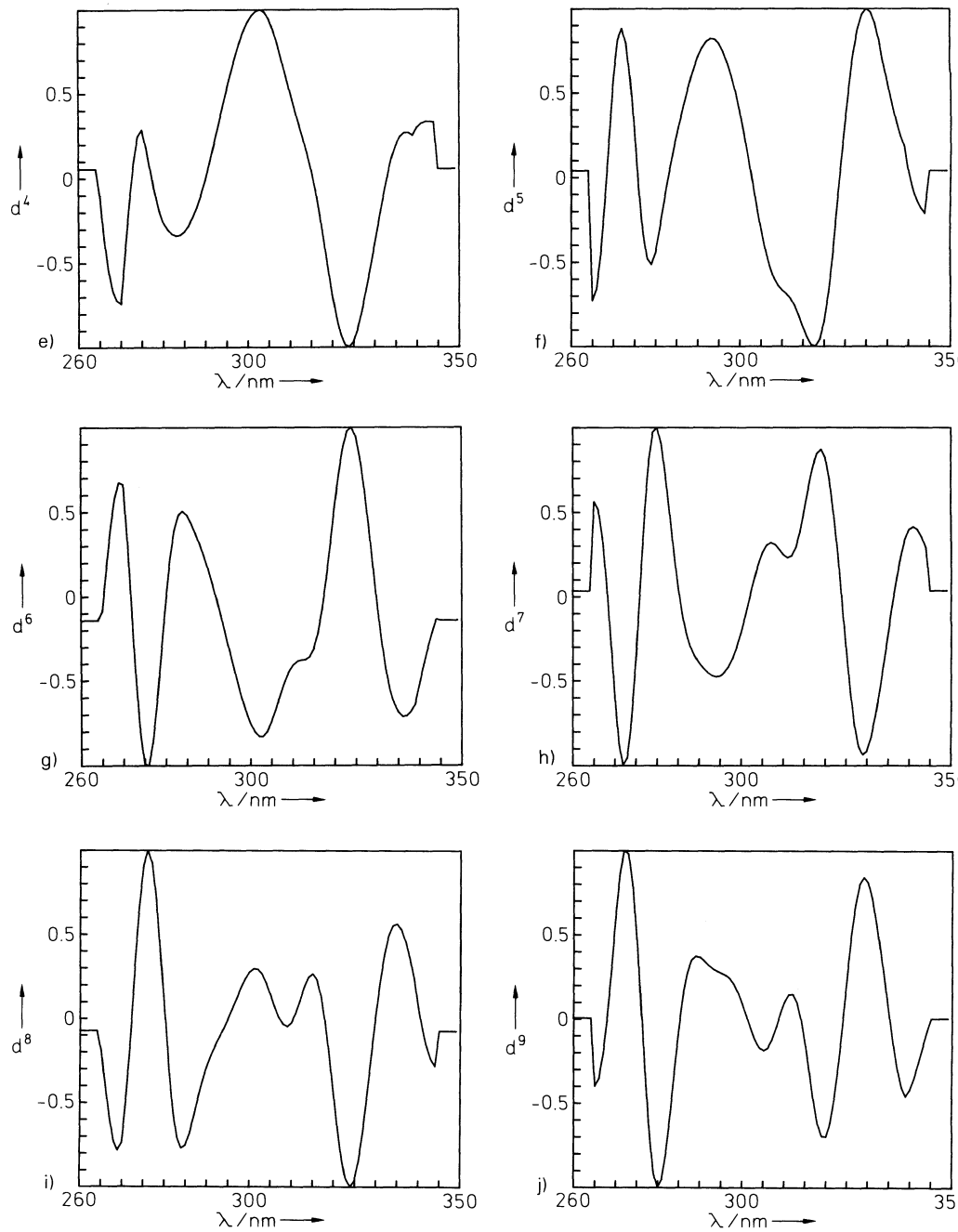


Figure 4-41. Potassium nitrate. Conc. 10 g L^{-1} in water (LTH: 1 cm, slit: 1 nm, scan speed: 2 nm s^{-1}, data interval: 1 nm, response: 2 s; MD: D (S-G 11).
a)-j): d^0-d^9 (all spectra normalized).

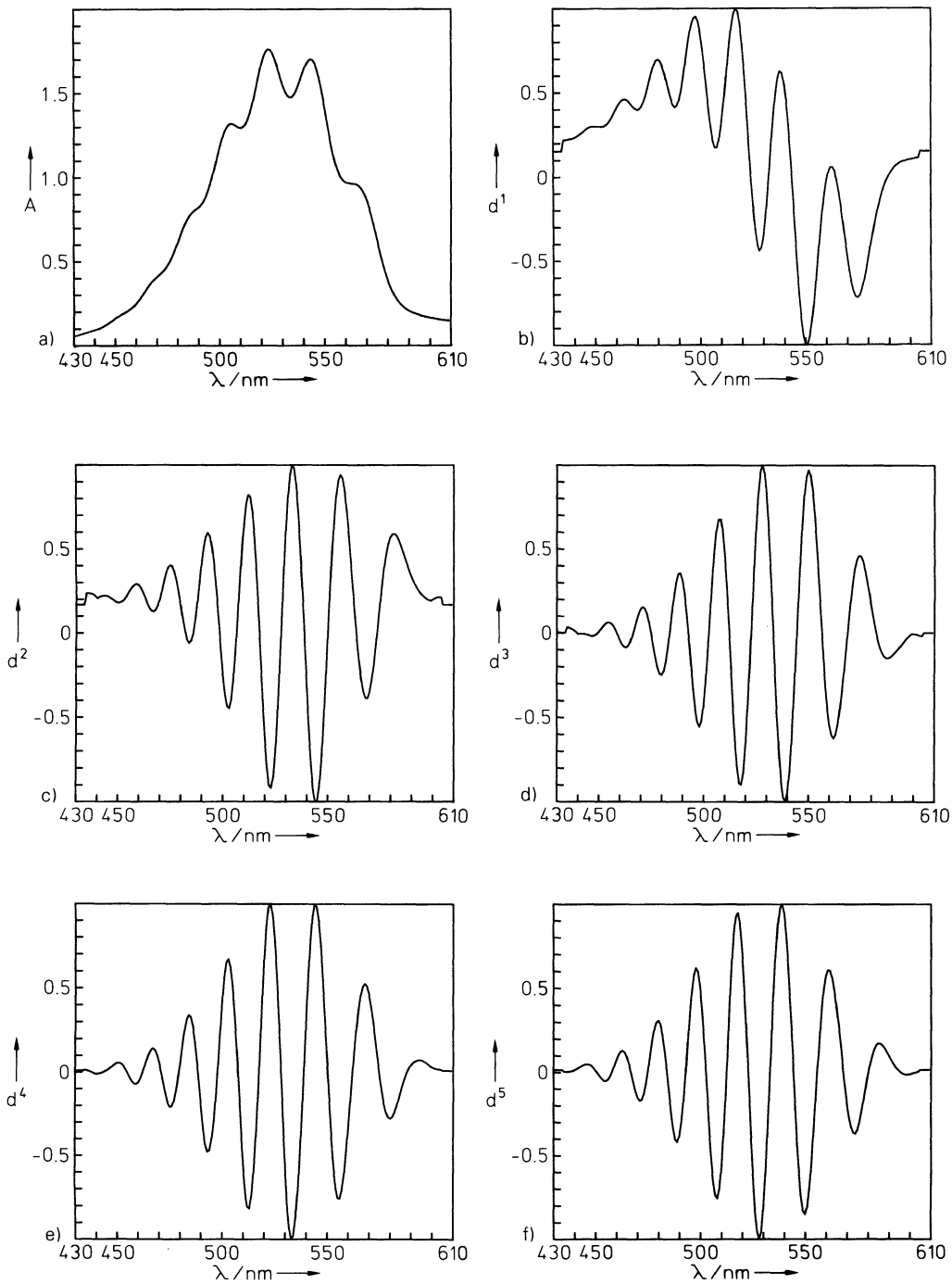


Figure 4-42. Potassium permanganate. Caption next page.

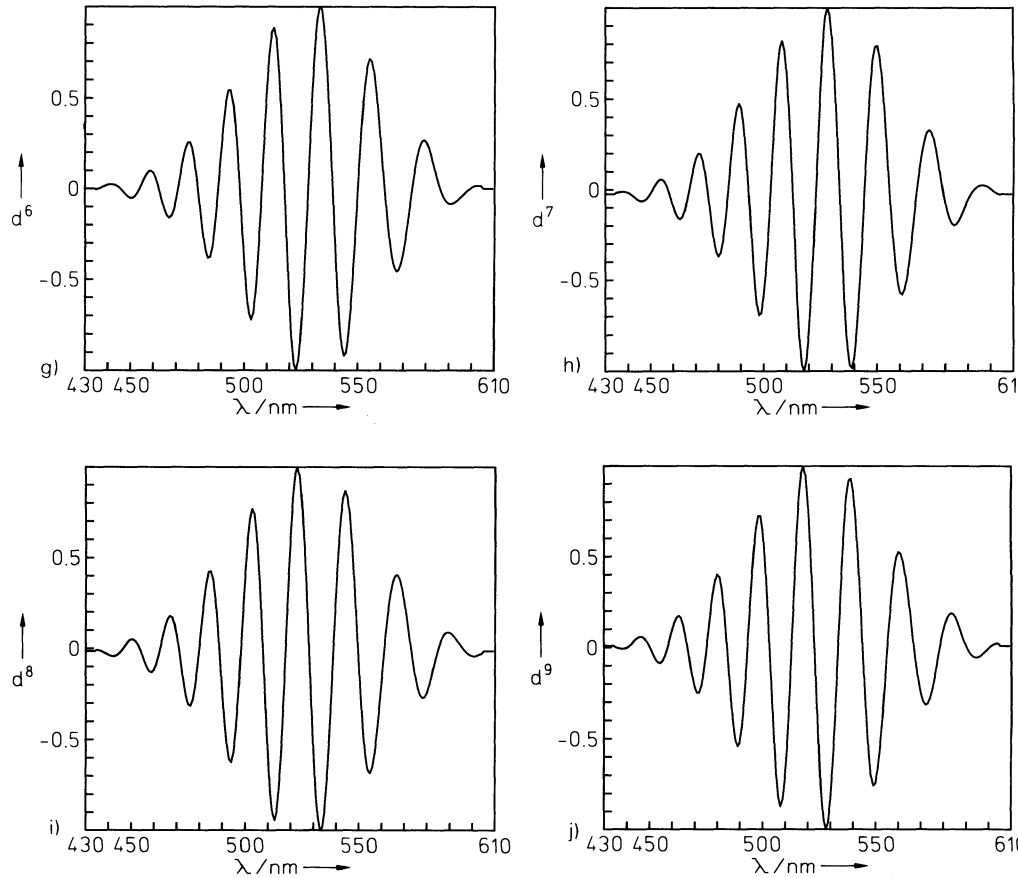


Figure 4-42. Potassium permanganate. Conc. 10^{-3} g L^{-1} in water (LTH: 1 cm, slit: 1 nm, scan speed: 2 nm s^{-1}, data interval: 1 nm, response: 2 s; MD: D (S-G 11). d^0 to d^9.

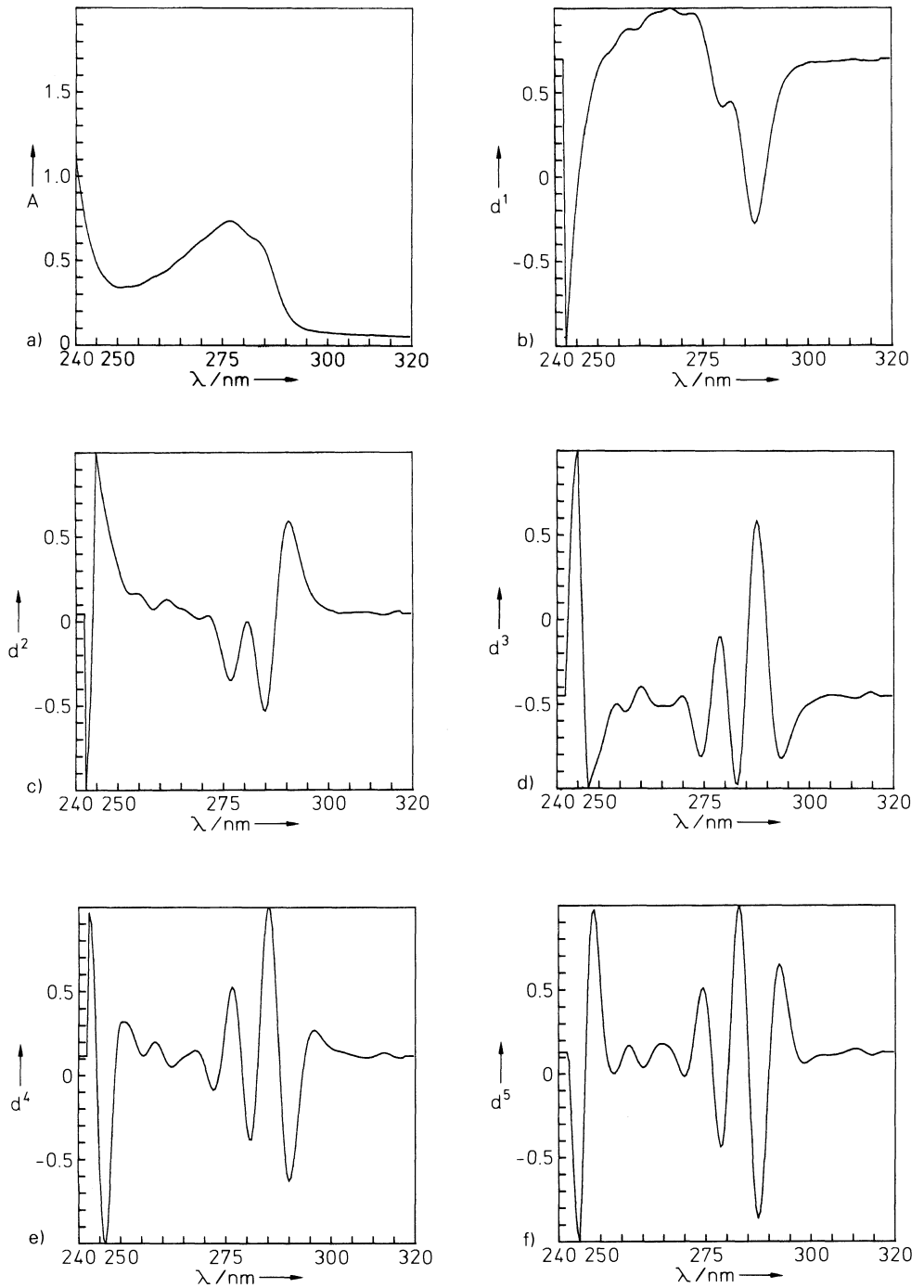


Figure 4-43. Bovine Ribonuclease. Caption next page.

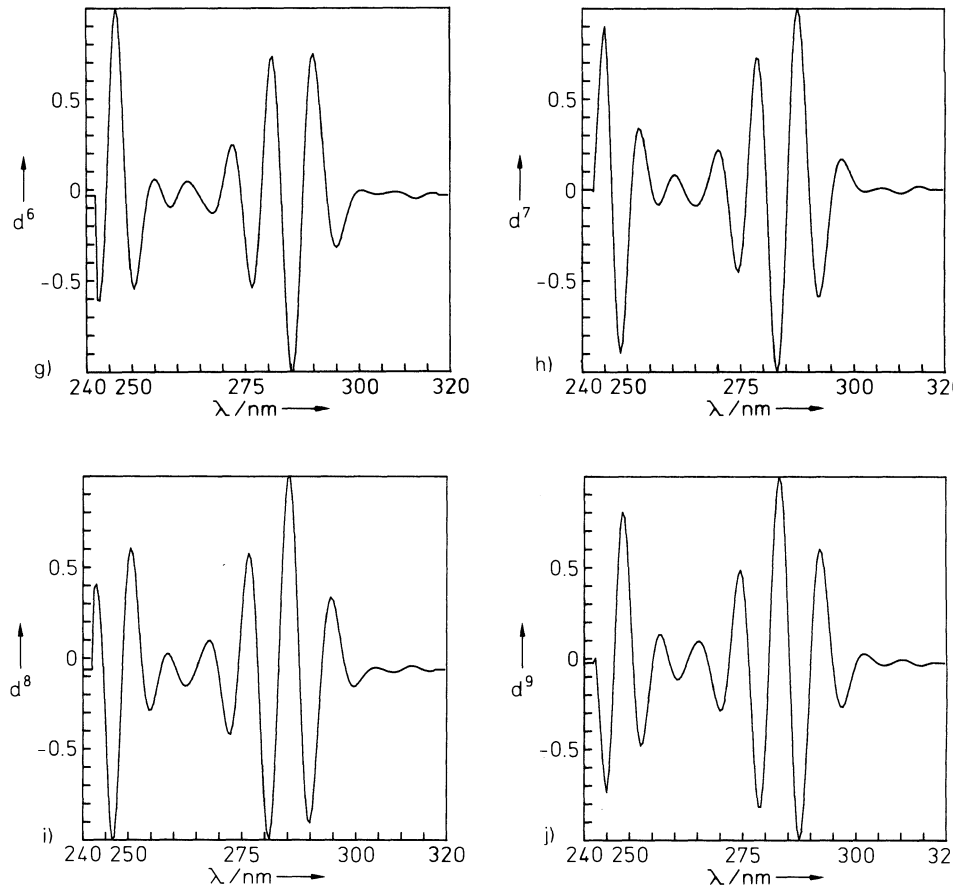


Figure 4-43. Bovine Ribonuclease, Conc. 1 g L^{-1} in water (LTH: 1 cm, slit: 0.5 nm, scan speed: 1 nm s^{-1}, data interval: 0.5 nm, response: 2 s; MD: D (S-G 11).
a)-j): d^0-d^9. Before differentiation, the fundamental spectrum was multiplied by a factor of 2. Spectra with a slit of 1 nm and a data interval of 1 nm gave unsatisfactory resolution. On the other hand, the spectra with a 0.2 nm slit and a 0.2 nm data interval contained too much noise.

4.7 Creating a Data Base of Derivative Spectra

Spectra catalogs are mainly limited to IR spectra, because the spectra of liquid or solid substances taken in the visible or UV region are mostly flat and only marginally characteristic. Higher-order derivatives are destinated to become the tool for making the fine structure of these signals more visible.

Above all, three points make the basis of organizing a data base of derivative spectra: the standardization of spectroscope technique, the normalizing of the deriv-

atives, and the availability of suitable software for the search program [33]. It must be kept in mind that not everyone has the most expensive high-performance spectrophotometer. Therefore, the normalized data have to be chosen so that as many spectroscopists as possible are able to work under the recommended conditions. It is of course possible to compare spectra generated by different derivative methods on different apparatuses under normalized conditions; their shapes should turn out to be similar. However, the best results are always attained by establishing one's own data base with spectra taken on the same spectrophotometer, because the fine structure should be always reproducible.

4.7.1 Normalization of Scan and Differentiation

In the foregoing sections we became acquainted with the influence of slit width, scan speed, number of data points, etc., on the resolution of the derivatives. Therefore, normalization of conditions is undoubtedly required if characteristic data of the spectrum is to be stored. In practice, the conditions compiled in Table 4-9 have proved to be a success [33].

In Table 4-9 there are two possibilities given for differentiation, namely, Δx for the difference quotient method, and the number of points for Savitzky-Golay polynomials. We prefer the first of these two methods because it is simpler and , nevertheless, very effective. Do not forget to correct, if necessary, all $\Delta\lambda$ shifts, in order to allow for correct estimation of λ positions of all maxima and minima.

Table 4-9. Recommended parameters for differentiation (see also [33]).

Parameter	Differentiation	
	Analog	Digital
Slit	0.5-1	0.5-1
Scan velocity S[nm s^{-1}]	2-5	1
f_c[Hz], filter (lowpass)	0.5-5	–
Response [s]	–	2-5
SNR	$\geqq 100$	$\geqq 100$
Maximal absorbance A of the fundamental signal	~ 0.8-1	~ 0.8-1
Δx (PP method)	–	1 mm $\triangleq$ 2 nm
or		
Number of datapoints (S-G method)	–	11-17
Height of derivative signal [mm]	~ 100	~ 100

4.7.2 Normalization of Derivatives

It is favorable to normalize all derivative spectra, if they have to be compared later by overlaying. This can be done by the multiplication method (see Sec. 4.6.1) which is mostly part of the common software for manipulation of spectra or by log A method (see Sec. 4.6.5) to be undependent of concentration.

4.7.3 Search Program

The data should be disposed hierarchically in a series of steps, which is proposed in Table 4-10.

At first, a search should be undertaken for all substances, for which the corrected λ of the maximum lies in a range of ± 5 nm around the λ (max) of the unknown sample. After that, the steps in Table 4-8 must be followed. By process of elimination, the number of possible candidates decreases to a few. Then the most probable spectra are compared with the unknown spectrum point by point on the video display computing the least-square error of the fourth derivative, or more simply, by the partitive method (Sec. 4.6.2), taking into account the deviations caused by the noise.

In the program given above, only third and fourth derivatives were used because most common modern photometers are now fitted out with devices up to this order. Moreover, in practice the bulk of the problems can be solved with such equipment.

As a rule, for the long-term storage of spectra forming a data base, generally floppy disks, recording tapes, or Winchester drives are used [16, 20, 34], in order to provide the fastest possible data activation.

Table 4-10. Steps in the proposed search program.

Step	Procedure
1	λ of maximum in d^4
2	λ of minimum in d^4
3	λ of 2nd maximum in d^4
4	λ of 2nd minimum in d^4
5	λ of maximum in d^3
6	λ of minimum in d^3
7	Comparison of spectra with standards
8	Determination of molecular formula
9	Determination of full chemical name

4.8 Comparison of Analog and Digital Differentiation Techniques

As stated in Chapter 3, only electronic modes are suitable for *higher-order* derivatives. Although digital differentiation techniques make up about 80–90% of all differentiation modes, it is nevertheless interesting to compare the advantages and disadvantages of both analog and digital devices [2–5, 11, 14, 16, 18, 34]. This was carried out in Table 4-11, in which the properties of the techniques are set against each other.

With respect to the quality of spectra, both derivative modes are comparable. This can be seen in Fig. 4-44. Of course, the spectra are not absolutely congruent – this cannot be expected –, because it is not possible for all parameters to be the same when established by different methods and different apparatuses. The main shape is similar,

Table 4-11. Comparison of analog and digital differentiation.

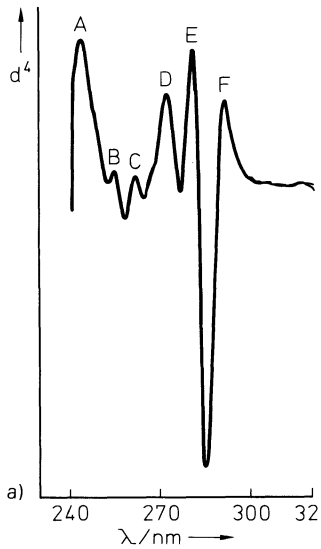
	Analog	Digital
On-line	Yes	Seldom
Off-line	Seldom	Mostly
Shift of spectra	More	Less
Handling	relatively simple	Knowledge of computer-technology or given algorithms required
Resolution (SSR)	can be very good	
Noise (SNR)	~100–200	
Price to performance	relatively low	Moderate
Filtering and smoothing	Relatively simple	Moderate to difficult
Distortion asymmetric	Low to Moderate	
Amplitude	Low to Moderate	
Optimization	Relatively simple	More complicated
Differentiation	$\frac{dA}{dt}\frac{1}{S} = \frac{dA}{d\lambda}$	$\frac{dA}{d\lambda}$
$\Delta\lambda$-correction (λ-shift)	Moderate	Mostly simple
Influence of scan velocity	Strong	Moderate
Influence of slit width	Evident	Evident
Longtime storage	Difficult	Relatively simple
Low-order derivatives	Simple	Moderate
Higher-order derivatives	Moderate	Moderate with special software
Signal manipulation	Moderate	Relatively simple
Amplifying	Automatically with differentiation	High amplification necessary
Simultaneous recording of more than one derivative	Yes	No (or very difficult by parallel operating PCs)
Quality of derivatives	Comparable	

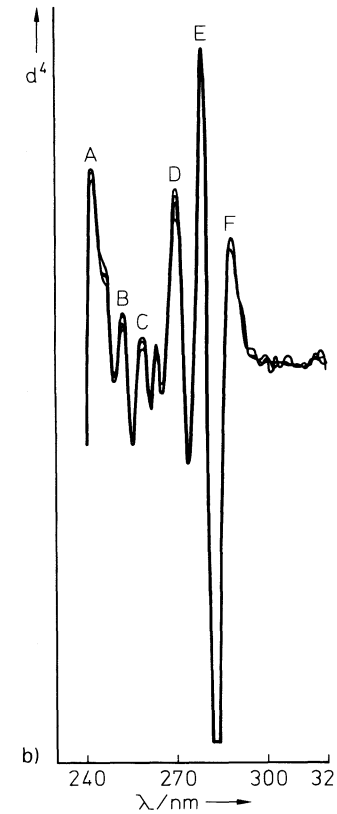
[a] *S:* scan velocity [nm s^{-1}]; *t:* time [s]; *A:* absorbance

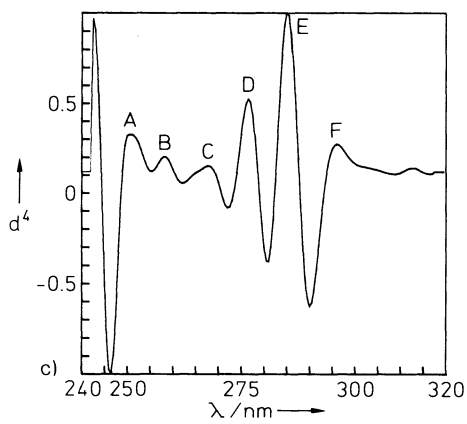
but the degree of resolution may differ. To corroborate this statement, we took the fourth derivative of the relatively stable protein ribonuclease I under different conditions and using different differentiation techniques. The individual spectra in Fig. 4-44a–b may now be discussed in detail.

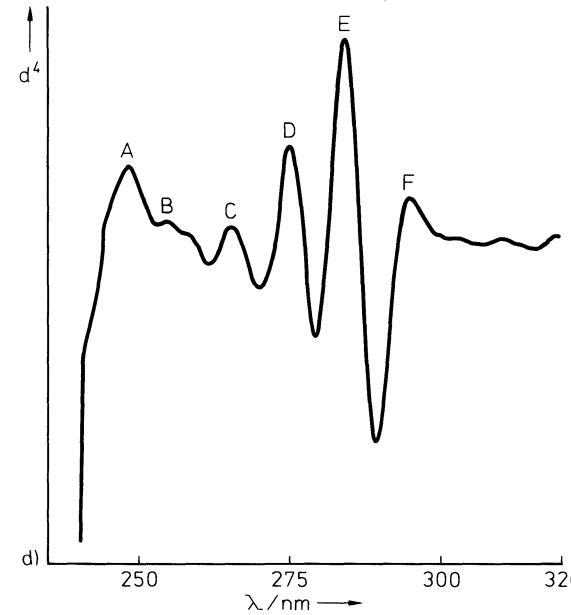
At first, an analog spectrum of RNase is given in Fig. 4-44a. In the UV region between 240 and 320 nm the fourth derivative shows 6 maxima (A–F). The superposition of 10 independent scans proves the excellent reproducibility of the signals. If higher resolution is desired, smaller time constants of the differentiators are necessary. In this manner, the shoulder of peak D in Fig. 4-44a is resolved in Fig. 4-44b, and on the asymmetric peak A, a shoulder is also obvious. On the other hand, the noise becomes visible if five independent scans are overlaid.

In Fig. 4-44c, the result of digital differentiation using the 11-point polynomial according to the Savitzky-Golay method is demonstrated. It is in good agreement with


d⁴
A
B
C
D
E
F
a)
240
270
300
320
λ/nm


d⁴
A
B
C
D
E
F
b)
240
270
300
320
λ/nm


0.5
0
-0.5
d⁴
A
B
C
D
E
F
c)
240
250
275
300
320
λ/nm


d⁴
A
B
C
D
E
F
d)
250
275
300
320
λ/nm

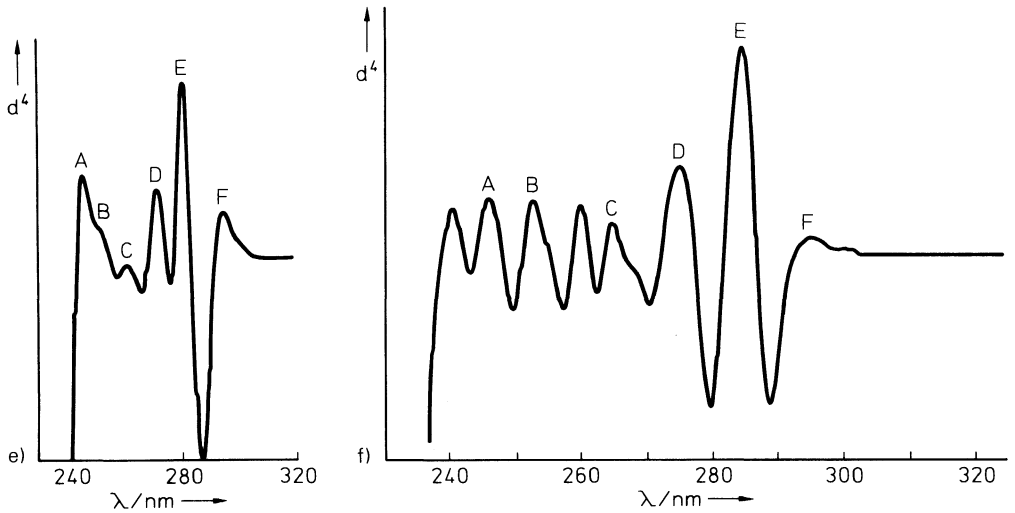


Figure 4-44. Comparison of the fourth derivatives of a bovine ribonuclease spectrum generated by different derivative techniques. Conc. 1.0 g L^{-1} in water.
a) Analog differentiation (LTH: 1 cm, slit: 1 nm, scan: 2 nm s^{-1}, range: 320–240 nm; superposition of 10 independent scans; b) analogous to a) with smaller differentiation time constant; c) digital differentiation (S-G 11), LTH: 1 cm, slit: 0.5 nm, scan: 1 nm s^{-1}, range: 320–240 nm, response: 2 s); d) Hybrid differentiation i.e., analog differentiation, digital smoothing and data manipulation); LTH: 1 cm, slit: 0.5 nm, response: 10 s; e) digital differentiation (point-point differentiation; difference quotient); LTH: 1 cm, slit: 1 nm, scan: 2 nm s^{-1}; point-point distance: 0.51 mm ≙ 0.54 nm. Between the differentiation steps, smoothing was carried out six times by a linear 5-point sliding window. f) digital differentiation (point-point differentiation). Analogous to e), but point-point distance 0.51 mm ≙ 0.2 nm.

the analog spectrum in (a) and also has 6 maxima, but it was necessary to choose a smaller slit width (0.5 nm instead of 1 nm) to obtain the same resolution.

Very similar to Fig. 4-44c is the course of the signal in Fig. 4-44d, generated by a hybrid technique. Recall that this is the mode with analog differentiation, digital smoothing operations (polynomials, response time), and other mathematical manipulations.

In addition, the result of the relatively simple point-point differentiation (difference quotient method) in Fig. 4-44e is comparable to (c) and (d). If the point-point differentiation distance of 0.54 nm is varied to 0.2 nm, then a better-resolved spectrum is obtained (Fig. 4-44f).

4.9 HODS of Opaque or Nontransparent Samples

The domain of spectrophotometric investigations of solids lies in the infrared range of the electromagnetic spectrum. The reason for this is the poorly marked UV–VIS spectra of opaque samples. Stray light results in a high loss of energy, and low to zero transparency leads to low residual energy reaching the multiplier of the spectrophotometer. This in turn leads to flat curves. The derivative mode is ideal for finely resolving such weak signals. The same is applicable to suspension and emulsions.

4.9.1 Absorption Spectra

4.9.1.1 Compact Solids

Thin plates, disks, or sheets of various materials, e.g., polymers, crystals, glasses, paper, cellophane, textile goods, leaves, or microscopic sections, are prepared for spectroscopic examination by being fixed between two frames that are fitted for use in typical sample holders. If the dimensions of the samples are very small, they must be positioned between two silica plates. Spectrophotometers that are fitted with a second sample holder directly in front of the end-on multiplier, (i.e., a multiplier located near the entrance of light in the silica window of the tube) are quite useful. Thus, the stray light can also be included in the detector.

4.9.1.2 Samples Adsorbed on Thin Layers

Particularly for microanalytical investigations, it is favorable to adsorb 1 to 10 μL of liquid or a few milligrams of a material dissolved in the same volume of water or another suitable solvent on thin-layers (0.2 mm) of alumina or silica stabilized on sheets of polystyrene, polyethylene, or aluminum [35].

Of course, developed spots of TLC can also be analyzed in the same manner [30]. The entire TLC sheets or cut-out spots are fixed between two silica plates; one of these plates has a cavity 0.2 mm deep to take up the sample. In Fig. 4-45 the powerful resolution of a spectrum of a TLC spot by HODS is clearly visible.

4.9.1.3 Powders

For investigations of powders two methods have been established being effective: In the first method, about 10 mg of powder are filled into the cavity between two silica plates (thickness of the layer 0.2 mm) [2, 17, 36–38]; the same procedure can be used for foils or gels, in which, for example, insoluble materials may also be embedded [2]. For the second technique, sample is diluted by mixing it with potassium bromide or potassium chloride in a micromill [2, 37] (3–30 mg sample in 300 mg medium) and then pressing tablets of 5.0 mm diameter (thickness: 0.20 ± 0.01 mm; pressure: 1.2 t (120 MPa), i.e., 60 kg mm^{-2}; pressure time: 120 s) as practiced in IR spectroscopy [2, 36]. It is preferable to use polyethylene or polyfluoroethylene powders (e.g., Hostaflon TF 9205,

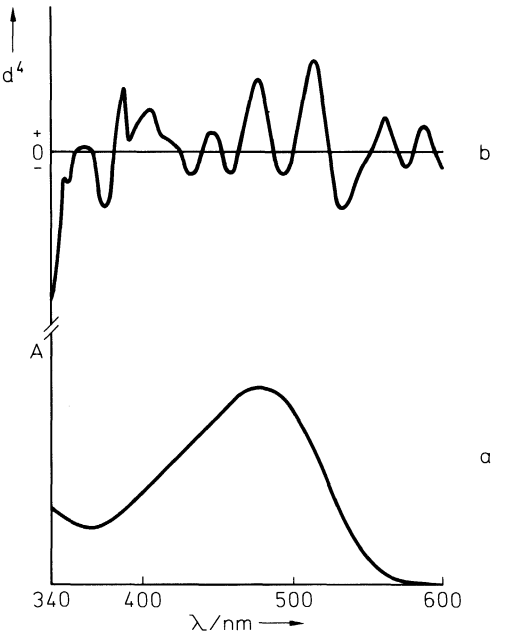


Figure 4-45. Absorption spectrum of pigment orange 5 (PO 5), adsorbed on a layer of silica with a thickness of 0.2 mm (TLC spot) (High energy, slit ~ 5 nm, scan speed: 2 nm s^{-1}, response: 5 s; MD: H. a) Fundamental spectrum; b) fourth derivative of a) [32].

Hoechst) for tabletting, because these substances are more inert than the potassium halides and do not react with the samples [37].

In Fig. 4-46 the spectra of a protein in solution, as a powder and diluted with KBr, are compared [2, 25, 36]. The character of the signals is identical; only the fine structure is slightly different, which is to be expected, because of the different environments of the chromophores.

4.9.1.4 Turbid Solutions, Suspensions, and Emulsions

Turbid solutions and suspended or emulsified solid or liquid materials make spectrophotometric investigations in the UV-VIS region much more difficult. Shorter wavelengths cause more light scattering, and the spectrum of the substances of interest are superposed by background, and the intensity of the signals goes down [2]. Investigating pigments in suspended yeast cells and chloroplasts, Butler and Hopkins [39-41] eliminated background with a function simulator and by calculating second and fourth derivatives by an off-line method. I made use of an analog differentiator for on-line quantitative estimation of phenol in wastewater which was turbid due to different concentrations of flocculating agents (silica, aluminum hydroxide). Nevertheless, the substance could be specified without being disturbed [42]. In the same manner other suspensions and emulsions could also evaluated [2]. The flow-through cell was positioned closely in front of the end-on multiplier, owing to scattering of light.

For stabilization of the suspension, substances that increase the density of the fluid and/or the viscosity may be added. Small amounts of sediments should be cen-

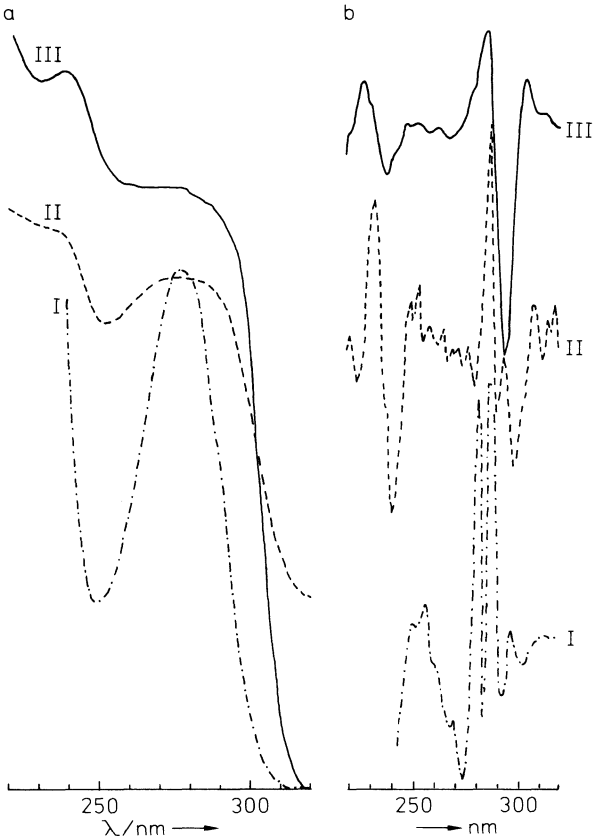


Figure 4-46. Bovine trypsin in solution (conc. 0.5 g L^{-1}) (I), as a powder (II), and in KBr tablets (0.2 mm) (III).
a) Fundamental spectrum;
b) fourth derivative of a);
MD: A.

trifuged in tubes fitted with a glass disk on the bottom, which is then fixed with the precipitate in the sample compartment for further spectroscopic investigations.

All measurements of opaque or turbid samples can also be carried out in an Ulbricht spheroid (see Sec. 4.9.2.1), which allows the estimation of integral stray light and absorption of the substance.

4.9.1.5 Frozen Solutions

Spectroscopic investigations with aqueous solutions below zero degrees celsius are particularly problematic because in most cases normal glass or silica cuvettes break when the water freezes. I used a special home-made vrass cell which enabled me to overcome these complications ([43-44] and Fig. 4-47).

The effective light path of the brass cell, which contains two silica windows, is 2 mm. To avoid condensation of water on the windows, the supports for the fiber cables are evacuable. The other end of both cables are connected by two optical adapters to the light source and to the multiplier of the spectrophotometer (Fig. 4-48).

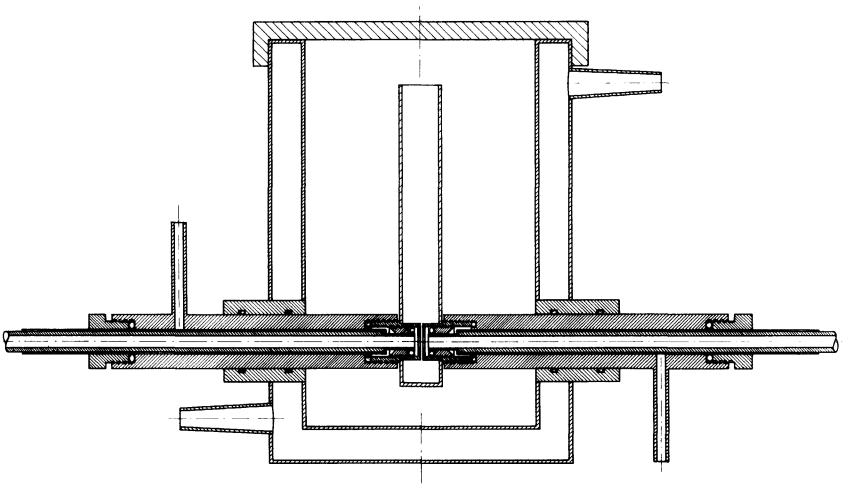


Figure 4-47. Photometric measuring cell for low-temperature and frozen solutions (Effective LTH: 2.0 mm; silica windows (Suprasil): 0.8 mm). The cuvette is connected by two fiber cables with the spectrophotometer and can be cooled or warmed. The space outside the windows can be evacuated to prevent humidity. For further details see [43–44].

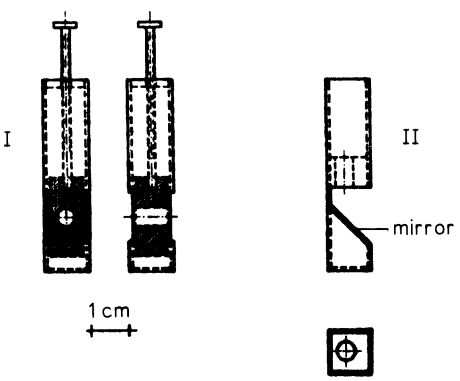


Figure 4-48. Adapters for connecting the low-temperature cell to the spectrophotometer by fiber cables. It is positioned in front of the outgoing light beam; II is positioned immediately in front of the multiplier. The mirror must be a true surface mirror [43, 44].

4.9.2 Reflectance Spectra

The same that was said for UV–VIS *absorption* spectra is also valid for *reflectance* spectra, which are primarily used for quantitative estimation at constant wavelength and not for taking a scan over a broader wavelength range. The signals are low in energy and are not very characteristic. Nevertheless, or perhaps for this very reason, HODS is very successful and therefore particularly recommended.

For practical work special optical devices are necessary. In this section we will cover the theory behind reflectance spectra and the use of these devices.

At first, some words should be devoted to the term *reflection*. Reflection occurs when light falling on a surface is thrown back in another direction. This is the reason why we are able to see matter, which is not self-emitting. In most cases the lighted surface becomes a starting point for radiation emitted in all directions – the light is scattered spherically into space. This *diffuse* reflection always occurs when the roughness of the surface is greater than the dimension of the wavelength (Fig. 4-49).

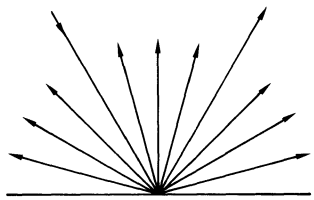


Figure 4-49. Schematic representation of diffuse reflection.

If the surfaces are very smooth, for instance surfaces of high-polished metals, glass, crystals, and also liquids, the incident beam is not thrown back diffusely but is reflected in one direction of the same angle and in the same plane. Such a phenomenon is known as *regular reflection, directed reflection,* or *specular reflection*. The angle of the incident beam is – in relation to a perpendicular line – the same as that of the reflected beam, that is, $\alpha_i = \alpha_r$ (Fig. 4-50).

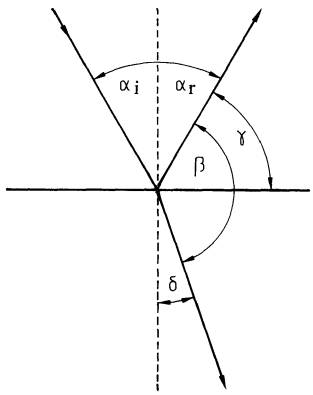


Figure 4-50. Reflection and deflection (schematic drawing) α_i: angle of incident beam; α_r: angle of reflected beam; γ: glossy angle; δ: angle of deflection: ($\alpha_i = \alpha_r$; $\beta = 180° - 2\,\alpha$; $\gamma = 90° - \alpha$).

In addition, *transparent* bodies reflect a part of the incident light at the entrance as well as at the point of exit. The other portion of light is refracted. In Figure 4-50 the incident beam is said to be *refracted* by the angle δ.

Regular reflection and integral diffuse reflection should only be considered as ideal cases. In practice, the manner of reflection lies somewhere between these two extremes. Even on an excellent mirror, the reflection point is seen, and a heavily frosted surface has a certain direction of regular reflection.

Diffuse reflection is a very complex process. Theoretically, it is assumed that reflected radiation is isotropic, which means that the radiation density is independent of the angle. If the size of the particles on the surface is of the same dimensions, as or is greater than the wavelength of the incident light ($d > \lambda$), one can imagine that the radiation is both reflected on the surface in all directions and also regularly reflected, refracted, deflected, and partially absorbed inside the particles [31].

Some important expressions may be summarized:

- Regular (specular) reflection: The angle of incident light flux is identical to the angle of reflection
- Diffuse reflection: Light is scattered at all angles from the point of reflection
- *Reflection factor (reflectivity):* Ratio of reflected light flux to the incident light flux
- *Reflectance (relative spectral directional reflectance):* Ratio of the radiant flux reflected from a light-diffusing specimen to that reflected from a light-diffusing standard.

In addition to the work of other authors (e.g., [46–49]), the best-known theoretical description of the diffuse reflection is undoubtedly the *Kubelka–Munk function* [50]. On account of the complexity of this effect, some simplifications must be carried out in order to facilitate a concise explanation:

- the surface is rough and is illuminated by diffuse light
- the particles of a given layer are completely disordered and much smaller than the thickness of the layer.
- possible parts of regular reflection are neglected
- the cosine law according Lambert (isotropical distribution of scattering) is applicable.

The Kubelka–Munk function $f(R_\infty)$ gives a relationship between the diffuse reflectance R_∞, the absorption coefficient k, and the scattering coefficient s.

$$f(R_\infty) = \frac{(1 - R_\infty)^2}{2 R_\infty} = \frac{k}{s} \tag{4-12}$$

R_∞ diffuse reflectance of the sample with thickness $d = \infty$
R_0 reflectance, if the ground of the sample is ideally black and not reflecting
R_g reflectance of the ground for $d = 0$
R reflectance of the sample for $d > 0$

Eq. (4-13) describes the relation between the diffuse reflectance and R_0, as a function of R and R_g.

$$R_0 = \frac{R_\infty (R_g - R)}{R_g - R_\infty (1 - R_g R_\infty + R_g R)} . \tag{4-13}$$

If $R_g = 1$, the ground is ideally white and R_0/R is the ideal contrast ratio. In practice $R_g = 1$ cannot be verified; MgO or TiO_2 may give a R_g of 0.98. With common

apparatuses it is impossible to measure R_∞ absolutely, but this quantity can be related to a white standard, and we obtain the relative quantity R'_∞ (*relative directional reflectance*)

$$R'_\infty = \frac{R_{\text{sample}}}{R_{\text{standard}}} . \tag{4-14}$$

The Kubelka-Munk equation is only strictly valid if the surface is illuminated by diffuse light and not by a directed beam [46]. For further information on these theoretical and practical aspects, see [51] and [52].

4.9.2.1 Integral Reflectance

The basis of the equipment for estimating the integral (total) reflectance is a hollow sphere or spheroid (*integral sphere*), which is coated with a layer of nearly completely reflecting flat material (about 96–98%), e.g., barium sulfate, magnesium oxide or polytetrafluoroethylene.

In order to truly integrate, the photomultiplier cannot "see" either the sample or spectacularly reflected light patches. The basic concept of the integrating sphere was first demonstrated by Sumpner in 1892 [53] and later studied in detail by Ulbricht [54] and others. Therefore such a sphere is generally called an Ulbricht sphere (Fig. 4-51).

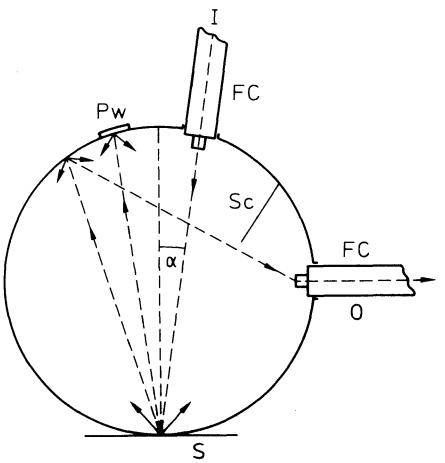


Figure 4-51. Ulbricht sphere for integral (total) reflectance. I: inlet port; S: sample; (positoned at the sample window); O: outlet port; Sc: screen (blocks the direct light path from the inlet to the outlet); P_w: white plate; α: angle of about 8° (schematic drawing).

The *integral (or total) reflectance* can be split in two components, i.e.,

$$R_i = R_d + R_r \tag{4-15}$$

R_i integral (total) reflectance
R_d *diffuse reflectance*
R_r *regular reflectance*.

The practical solution of the problem is obtained by modifying the equipment in Fig. 4-51. It is only necessary to exchange the white plate P_w by a black plate, or a *black light trap.* The regular beam is then absorbed and only the diffuse light reaches the output. A black light trap is a blackbody which absorbs practically all incident light. For HODS, diffuse reflectance is not as suitable as integral reflectance.

There are many variations of the principle of integrating spheres for both the monobeam mode and the double-beam mode. An example of the latter is given in Fig. 4-52.

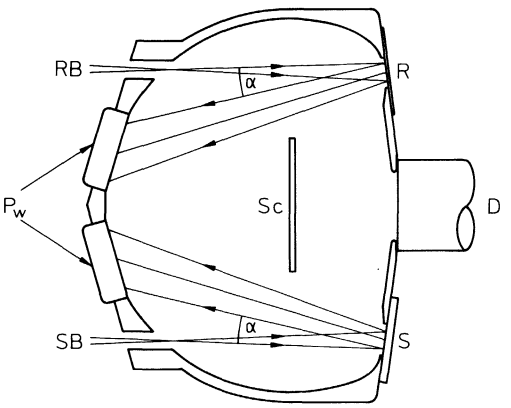


Figure 4-52. Dual-path spheroid. RB: reference beam; SB: sample beam; R: reference; S: sample; D: detector (multiplier); Sc: screen; P_w: white plate for integral reflection (or blackbody for diffuse reflection); α: angle of about 8° (similar to [55]) (schematic drawing).

Integrating spheres or spheroids can be used between about 250 and 2500 nm with maximum levels from 400 to 1100 nm, depending on the coating material. Moreover, it should be noted that the total area of all ports does not exceed more than 10% of the total inner area of the body. This has to be taken in consideration when choosing the inner diameter of the sphere or spheroid.

Various other design parameters are used to describe the performance of an integrating sphere. One of these is the *throughput t,* i.e., the ratio of flux exiting the sphere to that entering the sphere [56]:

$$t = f_e p/[1 - p(1 - f_t)] \tag{4-16}$$

where

f_e (area of exit port)/(area of sphere)
f_t (total area of all ports including exit port)/(area of sphere)
p spectral reflectance of sphere coating.

The total flux reaching the exit port is uniformly distributed over the total area of the exit port.

Generally, reflectance R (in German literature mostly called *Remittance*) is given as a percent related to a white standard (e.g., barium sulfate, metallic gold, polytetrafluoroethylene). This is comparable with the transmittance T for investigating transparent samples; in both cases, the variable represents the *nonabsorbed* energy. In Sec. 2.2

we saw that in taking the derivatives, it is preferable to differentiate the absorbance A rather than the transmittance T. Therefore, in analogy to

$$A = \log \frac{1}{T} = \log T^{-1} \tag{4-17}$$

we can take the *reflective absorbance* A (Ref).

$$A\,(\text{Ref}) = \log \frac{1}{R} = \log R^{-1} \tag{4-18}$$

In this special case, R is equal to R'_∞, the relative directional reflectance (see Eq. (4-14)). The comparison of differentiated absorption spectra with differentiated reflection spectra is then easier, and they are also more clearly arranged [31]. To demonstrate this, in Fig. 4-53 the fundamental spectrum and the fourth derivative of a wheat leaf are given in terms of absorbance and reflectance [57–58]. The shape of the curves are similar, and only the A (Ref) spectrum is slightly shifted to longer wavelengths.

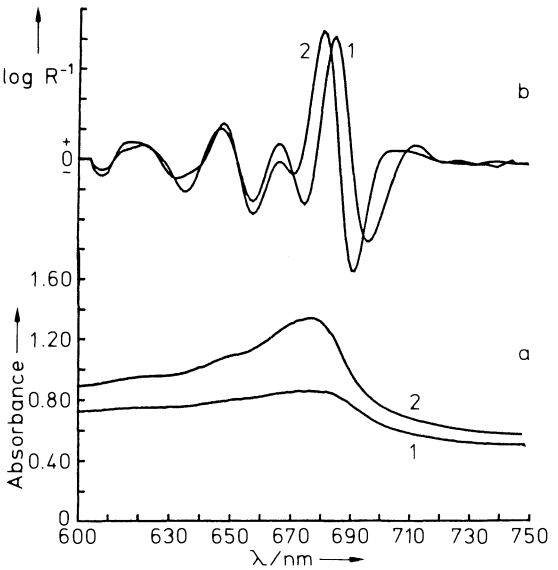


Figure 4-53. Comparison of absorption and reflection.
a) Fundamental spectrum of a wheat leaf in terms of absorbance (2) and reflectance (1); b) fourth derivatives of a) [57, 58].

4.9.2.2 Regular Reflectance

For investigations in the IR region, a great number of different devices have been constructed. In principle, the light beam coming out of the monochromator is directed onto the surface of the solid by a certain angle with mirrors or prisms, and the regular reflected beam is turned back to the detector. In some cases, this equipment can also be used in UV–VIS spectrophotometry. As an example, such a device is depicted in Fig. 4-54.

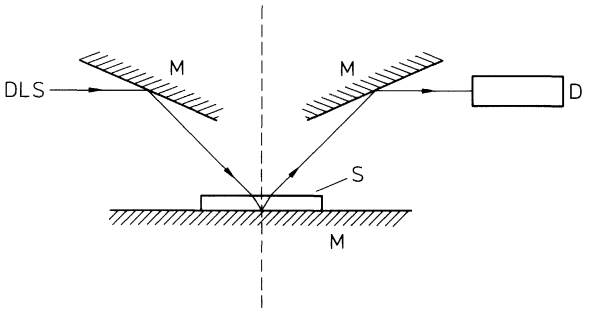


Figure 4-54. Device for regular reflectance. DLS: directed light source; M: surface mirror; S: sample; D: detector (schematic drawing).

In principle, these devices produce absorption spectra; the sample is illuminated twice. Using UV–VIS sources with shorter wavelengths, some of the incident light is also absorbed, the rest of it is not only regularly reflected but also diffusely scattered, which causes a loss of energy, because only the specular reflected beam reaches the detector.

In Sec. 4.9.2.3 some proven devices for UV–VIS reflectance will be treated.

4.9.2.3 Flexible External Measuring Devices

All attachments to spectrophotometers discussed previously must be set up in the sample compartment. This may sometimes be obstructive, expecially if the sample is large. In such cases, the sample compartment is too small to insert larger objects, and it is often not possible to cut off small pieces for investigations, for instance, when the objects are living organisms which may not be disturbed or killed. A flexible external device that enables one to lead the light source "around the corner" can solve the problem in an elegant and very practical manner.

Preparation of samples is in most cases unnecessary. What is needed are flexible fiber optic cables for light conduction, adapters and optical measuring heads.

Fiber Optic Cables

The principle of the *Fiber optic cable* is based on the total reflection of a beam at the boundary of a phase with a high refractive index and a phase with a low refractive index (Fig. 4-55). If the cable is bent, the beam nevertheless remains in the inner portion of the fiber (Fig. 4-56).

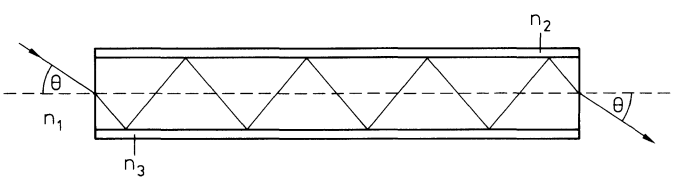


Figure 4-55. Principle of total reflection in a fiber optic cable. n_1 refractive index of the medium of the surroundings (e.g., air), n_3 refractive index of the jacket of the fibers, n_2 refractive index of the fibers, Θ angle of the incident beam. $n_1 < n_2 < n_3$ (schematic drawing).

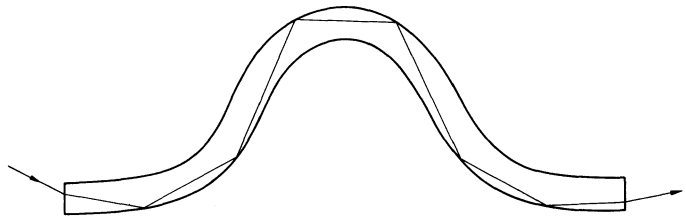


Figure 4-56. Light conduction by total reflection in a monofiber.

The most important characteristic quantity is known as the *numeric apperture, NA:*

$$NA = n_1 \sin \Theta = \sqrt{n_3^2 - n_2^2} \; . \tag{4-19}$$

NA describes the critical angle of the incident beam which limits the transport of light in the mono fiber. The fiber optic cables on the market usually consists of a bundle of mono-fibers the diameter of which ranges from 70–100 μm. Cables having only one thicker monofiber are less flexible.

In order to connect the cables onto the spectrophotometer, two adapters are necessary – one for the outgoing beam and one for the returning beam. In 1981, I made use of a home-made device [43],which had the dimensions of a normal 1 cm cuvette and could be positioned in common sample holders without additional modifying of the spectrophotometer (Fig. 4-48).

Böhme et al. [59] used mirror optics, in which the returning beam was focused by a spherical mirror (radius 108 mm) on the detector.

A disadvantage of fiber cables and other optical devices is that they bring about a loss of intensity. Depending on the fiber material, cable length, and wavelength of incident light, this loss can vary between 75 and 100%. If the fibers are made of glass, not enough energy is available below 400 nm to obtain satisfactory derivatives. With silica fibers, the borderline of applicability is shifted to about 250 to 300 nm (Fig. 4-57 and 4-58).

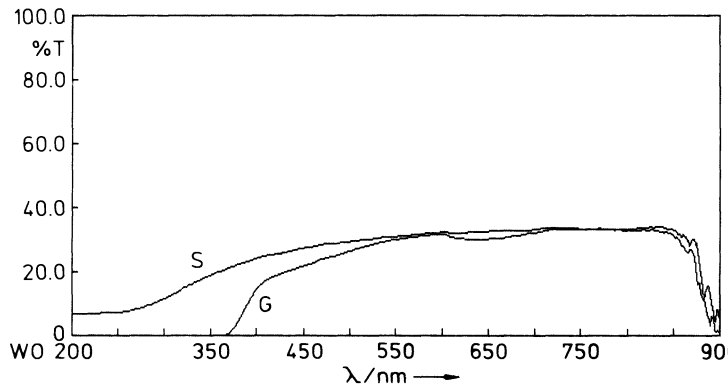


Figure 4-57. Transmission spectra of fiber cables. G: 120 mm glass fibers (ø3.2 mm), S: 60 mm silica fibers (ø2.8 mm).

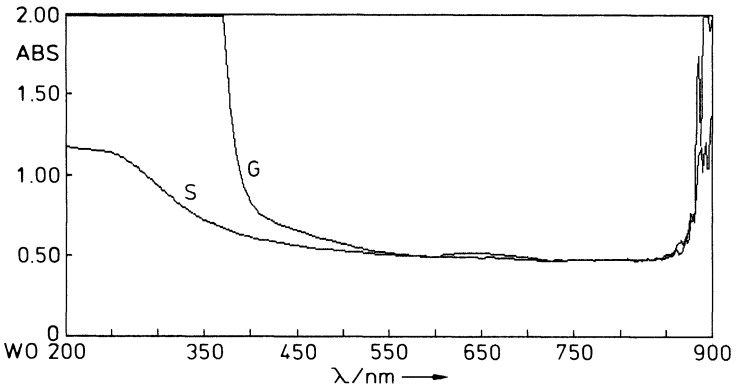


Figure 4-58. Absorption spectra of fiber cables. G: 120 mm glass fibers, S: 60 mm silica fibers.

The dependence of absorption on the fiber cable length is given in Fig. 4-59. It appears that the absorption rises nearly linearly to the length of the fiber cable.

In order to compensate this difficulty, the slit width must be 1 nm or greater and, if necessary, spectra must be accumulated. This is especially important if the data are be worked up to generate higher-order derivatives.

If double-beam spectrophotometers are used, a 10% T or 1% T optical attenuator (hole sieve, grey filter, opalescent glass), or preferably the same optical arrangement (fiber optic cable, lenses, adapters, etc.) must be taken.

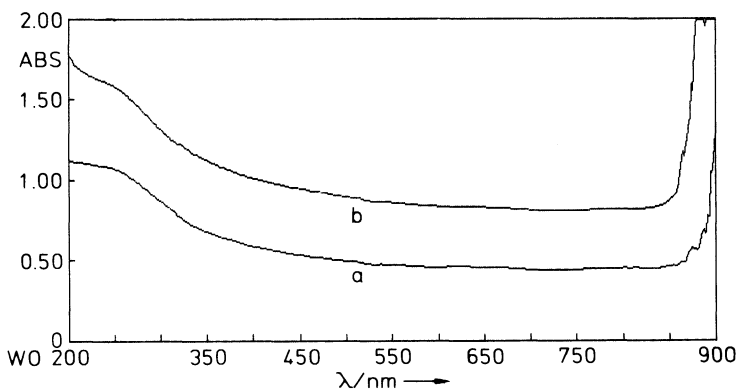


Figure 4-59. Absorption spectra of silica fiber cables with different length.
a) length 60 mm; b) length 120 mm.

Measuring Heads

Measuring heads must be accommodated to the experimental conditions. For example, I used a low-temperature cell for studying the kinetics of an enzyme-catalyzed reaction in a frozen aqueous medium below 0° [43], and Y fiber optic cable for 0°/180° reflectance measurements [31], and an Ulbricht sphere (∅ 20 mm, Fig. 4-60) for integral reflectance measurement to investigate synthetic art paints [31–32].

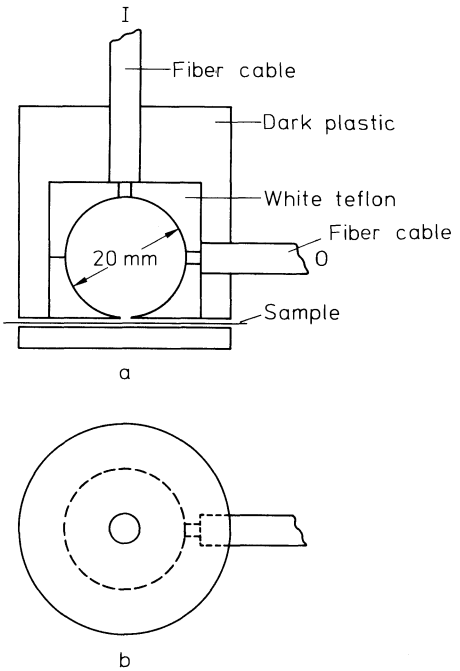


Figure 4-60. Ulbricht sphere for integral reflectance.
a) Sectional side view; b) view from above; I: inlet; O: outlet.

For directed reflectance, relatively simple adapters give very good results [57]. Some types of measuring heads are summarized in Fig. 61. They are made from a black (or grey) polymer.

It became apparent that angles of 60°/60° led to worse results. Between 30°/30° and 45°/45° no different behavior was observed.

Likewise, simple supports were also described by Horn [60] for transmission and absorption measurement and are shown schematically in Figs. 4-62 and 4-63.

In order to use these supports for reflectance measurement, an additional optical arrangement is necessary and must be positioned between the fiber holders or lenses. Moreover, it must be covered with a dark cloth.

Further references:

- apparatuses for reflectance [61]
- transfer optics [59, 62–63]

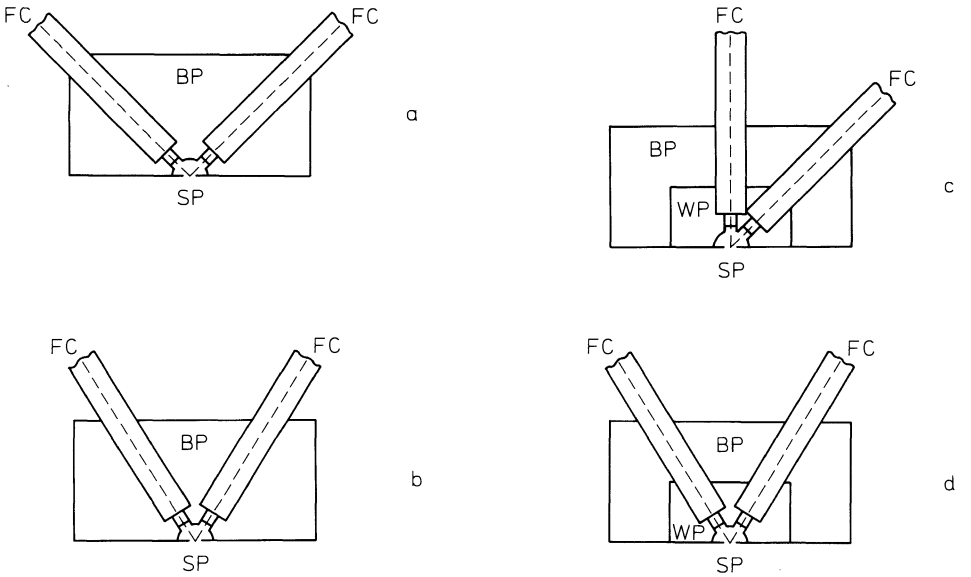


Figure 4-61. Different measuring heads for different modes of reflectance (cross section). a) 45°/45°: regular reflectance; b) 30°/30°: regular reflectance; c) 0°/45° diffuse reflectance; d) 30°/30°: integral reflectance. BP: black plastic; WP: white plastic; FC: fiber cable; SP: sample port.

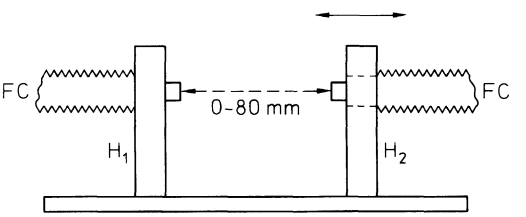


Figure 4-62. Simple fiber cable support (after [60]). FC: fiber cable; H_1, H_2: movable supports for fiber cables. The sample is positioned between H_1 and H_2.

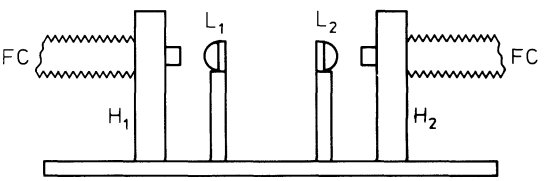


Figure 4-63. Improvement of cable support from Fig. 4-62. FC: fiber cable; H_1, H_2: cable supports; L_1, L_2: converging lenses for light focusing (diameter: 20 mm, $f = 25$ mm). The sample is positioned between L_1 and L_2 (after [60]).

- optical sensors [64–65]
- sample holder [61, 66]
- Ulbricht sphere (external) [67–68]
- "Praying Mantis" and other reflectance devices [69].

4.10 Guidelines for Generating Derivative Spectra

In conclusion, the most important points to remember for generating derivative spectra are summarized here in the form of twelve guidelines. Obey these instructions strictly if you want to avoid mistakes.

1. Start the derivative operation by filtering or smoothing the rough data of the fundamental signals. Carry these operations out only as far as is absolutely necessary.
2. Filtering or smoothing always involves a compromise between resolution, noise (SNR), and distortion of the signal.
3. If it is practicable in some way, accumulate signals many times. This is the best method of smoothing without deforming the shape of the signals.
4. Smoothing by a "sliding window" is more effective if you take a few data points several times rather than many points only once.
5. If the fundamental signals are of low intensity you must amplify them *before* filtering or differentiating.
6. Differentiate *step by step* (e.g., it is better to take four first-order derivatives than to take one fourth-order derivative). This is valid both for analog and for digital techniques.
7. If necessary, smooth between each differentiation step as well.
8. The best test for distinguishing whether the peaks are genuine or are artifacts caused by noise or other disturbing factors is the comparison of independently scanned signals under equal conditions.
9. Remember that *maxima* give extrema in even derivatives, and zero crossing in odd derivatives; *inflection points* give zero crossing in even derivatives, and extrema in odd derivatives.
10. Optimization of smoothing and differentiation parameters and of the differentiation order is reached if all shoulders and inflection points are resolved and if the signals oscillate around the zero line.
11. Generally, the limit of multicomponent analysis is reached with three components if you want to estimate all substances quantitatively. Otherwise, it is possible to estimate some components quantitatively in a mixture of many other substances by standard addition technique.
12. The full information lies *exclusively* in the fundamental spectrum. Therefore, the derivative technique cannot give more information, but derivatives, especially higher-order derivatives, make this information more accessible, more visible, and easier to evaluate.

4.11 Present and Future Perspectives

The evaluation of more than 1200 references concerning derivative spectrophotometry up to the first half of 1991 gave very interesting results. From the beginning in 1951 up to 1965 only a few publications are found in the literature. The first modest accumulation

of articles appeared in 1970. From 1975 to 1982 the number of papers grew to about seventy publications per annum and reached a plateau between 1983 to 1985. The number has continued on rise. For the year 1990, for instance, 141 papers were registered (Fig. 4-64).

Whereas between 1951 and 1975 most papers dealt with theoretical issues or equipment problems, in the following years more and more publications were concerned with analytical applications. Currently, this excellent method is being widely used not only in scientific laboratories but also in industry and in national bureaus of standards.

What can we expect in the future? Surely, the use of digital differentiation will further increase, but not because of better differentiation quality. This can be shown by comparing many examples. The reason for this evolution is the digital trend and the fact that most apparatuses are connected on-line to a computer or contain an integrated computer. Moreover, the most-modern spectrophotometers and other devices offer digital data storage and as a rule do not have any analog output. Perhaps some variations of algorithms for reducing data, smoothing, and differentiation will be developed, but it is scarcely to be expected that a totally new method will emerge. The crucial point lies in the multifarious applications of this analytical technique.

Especially in UV–VIS spectroscopy, improved diode-array spectrophotometers or other fast spectral devices will be favorable for the fine resolution as well as the enhacement of sensitivity of spectra and other electric signals. By rapid accumulation of many scans, the best method for noise elimination can be employed from a rational point of view, and in the shortest amount of time, without altering the shape of the signals.

In the foregoing years the number of spectrophotometers that are able to generate derivatives of fourth or higher order also grews. For this reason, it will be expected that the advantages of higher-order differentiation, or multidifferentiation, will also be recognized, and that the potential for better exploiting the information existing in fundamental signals will be put to use still more frequently.

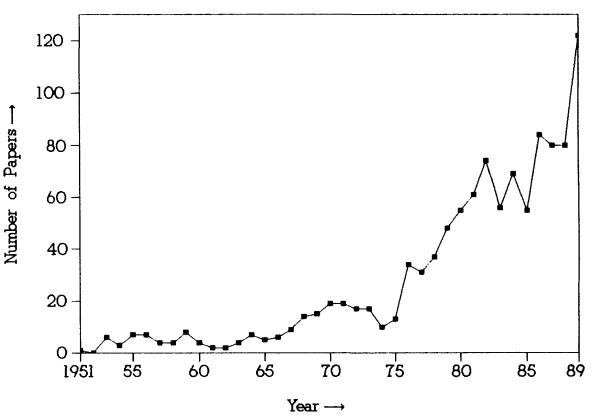


Figure 4-64. The number of papers concerning derivative spectrophotometry and other derivative modes from 1951 to 1989.

4.12 References to Chapter 4

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5 Applications

Most of the approximately 1200 papers on derivative techniques (up to July 1991) deal either with applications of this method to finely resolving signals or with applications of theoretical considerations to practical problems. At the moment about 110 to 140 new papers are presented per annum.

In this chapter 489 examples of applications are carefully selected out of the large number of publications. The literature is summarized in groups of different substances or fields of applications. We hope this information may encourage the reader to adopt higher-order differentiation in analytical or other problems and will provide an useful introduction to the literature.

In the following review application for spectroscopic and nonspectroscopic fields along with comments, all based on the important references and explanatory practical experience of the author are found in 57 tables.

5.1 UV-VIS Spectrophotometry

Previously, UV-VIS spectrophotometry was used preferably for quantitative estimations of concentrations of known substances at constant wavelength, because the fundamental spectra are mostly flat and are less characteristic than IR spectra, for example. However, higher-order derivatives now allow for an enhancement of the sensitiveness by a factor of 10-100 or more as well as a characterization of the substances by providing fingerprints, even in complex mixtures. This is very important for ultramicroanalysis. Therefore, the bulk of papers concerning differentiation technique deals with UV-VIS spectra. It is also the reason why in this book the field of UV-VIS spectra is treated in detail.

5.1.1 Inorganic Cations

A number of cations of the transition elements were investigated as well as some others of the main series. Most of them absorb in the visible region. Those which are colorless in the visible range of the spectrum were colored by complexation with suitable reagents

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(mainly organic reagents) or investigated in the UV. The estimation of different cations in water, soil, or air can be found in the section on environmental analysis.

For quantitative investigations, standard lines are necessary, or the standard addition method must be used. The evaluation of the derivative peaks is made by measuring the peak height according to the PP, PZ, or PT method or by estimation of the peak area. After this test, the technique which gives the smallest error deviation should be chosen. If the derivative spectra have more than one maximum, not all peaks are suited for evaluation; it may be that the one of them is superposed by satellites which can lead to deviations in the linearity of the standard curves.

Generally, not more than three to four components are simultaneously estimable by HODS, but mostly it is generally not detrimental to have a number of other unknown substances contaminating the sample.

The combination of HODS followed by the multicomponent calculation technique can sometimes be advantageous for separating strongly overlapping signals.

It is remarkable that a great deal of papers concern the identification and estimation of the lanthanides. Certainly, a contributing factor to this circumstance may be at these elements are highly similar to each other, and that fine resolution of derivative spectra is feasible, thus facilitating the investigation of purity.

In Table 5-1 to 5-4 some references and comments are made concerning the quantitative estimation of different cations.

5.1.2 Inorganic Anions

In contrast to cations, there are only few publications dealing with quantitative estimations of anions. In particular, the oxy acids of the elements B, Cr, Mn, and P were investigated. Otherwise, all statements made concerning cations are valid for anions as well (Table 5-5).

5.1.3 Minerals and other Inorganic Solids

Inorganic solids have not been investigated extensively by derivative spectrophotometry. Optical glass filters, transparent crystals of different inorganics, and gems cause no problems. More difficult to examine are thin films of opaque powders. In this case, it is also possible to press thin tablets of a mixture of the sample and a suitable diluting substance such as KCL, KBr, polyethylene, polyfluoroethylene and others. Less than 1 mg of the sample is enough for this analytical technique. It is also possible to investigate suspensions of the solids in a fluid; additives which raise the viscosity can be included to prevent sedimentation of the particles. Particularly with low-energy spectra and strongly scattering samples, the advantages of the HODS are evident. Some examples are found in Table 5-6 (see also Table 5-42 on reflectance spectroscopy).

5.1.4 Gases

Gases can also be investigated by HODS (Table 5-7). If the absorbance is very weak, it is better not to use a common 1 cm cell but rather a special device which enables the light beam to pass through the chamber several times by reflection with mirrors. In this manner the cell pathlength is elongated and the absorbance becomes higher.

5.1.5 Organics

The focal point of applications in organics lies in aromatic amino acids, proteins such as enzymes, pharmaceuticals in various forms, and natural (native) and synthetic pigments and dyestuffs.

The relatively high proportion of investigations on proteins (and amino acids) has its main origin in the highly sensitive variation of spectra which occurs by changing the pH or the salt concentration and by adding other substances to the solutions, e.g., organic solvents. Shifting of the maxima as well as variation of the absorption intensity can be observed if the surroundings of the aromatic chromophoric amino acids are altered. Therefore, it is difficult to find a *general* standard for quantitative calibration in all situations. For this reason the analysis must be adapted to the required conditions. On the other hand, it is possible to study conformational changes by comparing spectral changes on account of the high sensitivity of the method (Table 5-9, 5-17, 5-30, 5-48).

The last point was the main reason why my co-workers and I became aquainted with the derivative technique in 1976, studying the effect of chemical modification of enzymes. First, we developed an analog second-order derivative module, and later created home-made devices for generation of low-noise derivatives up to the ninth order (ADT 2, ADT 7, ADT 9; TLB 6000), in order to systematically evaluate the advantages and disadvantages of the higher-order derivative technique ($n > 2$). Finally, we made use of different digital modes of signal differentiation comparing the results of various algorithms.

In their investigations of highly complex materials, biologists quickly realized the advantages of the derivative technique. It is thus not surprising that a number of papers deal with spectra of chlorophylls and other plant pigments as well as with chlorophyll-protein complexes (Table 5-11).

Both, natural pigments and synthetic organic pigments and dyestuffs have been treated in numerous publications. The fine-resolution of spectra by differentiation makes this technique a particularly powerful tool for identification and for verifying quality and purity (Table 5-16 and 5-26).

Derivative spectra of foods and beverages have also been taken. They help to ensure that the strict quality control specifications for such products are met (Table 5-14 and 5-18).

Very frequently, the derivative technique has been used for investigations and quantitative estimations of many pharmaceuticals in different media and formulas, mostly without special preliminary treatment of the samples. This has proved to be a very effective and time-saving procedure (Table 5-25).

Finally, it may be pointed out that substances such as phenols, halophenols, polynuclear aromatic and heterocyclic compounds are well suited for micro and

ultramicro estimation by HODS. This is very important for environmental investigations (Table 5-35).

Other groups such as vitamins and polymers have been investigated by HODS less frequently, but they are no less interesting for this reason (Table 5-34 and 5-28).

5.2 Other Spectroscopic Methods

One of the first applications of the derivative mode in spectroscopy involved infrared and atomic absorption spectroscopy (Table 5-36 and 5-38). Today, UV–VIS applications are clearly dominant, because most IR and AA spectra show distinct peaks and only rarely have unresolved shoulders.

In luminescence spectroscopy, derivatives have been used relatively frequently (Table 5-39). In this case, the high sensitivity of this method could be further enhanced. Similarly, the weak signals of reflection spectra are much easier to evaluate after multidifferentiation (Table 5-42). Nearly all other spectroscopic techniques are covered in the literature but up to this date the number of papers is still small.

5.3 Special Fields of Application

In this section special fields of application are summarized, among which analysis of biological materials, clinical chemistry, environmental analysis, conformational analysis, and microanalysis predominate (Table 5-46, 5-47, 5-48, 5-49, 5-53). The high sensitivity of the derivative technique as well as the possibility of direct investigation of the samples without special preparations and the effective removal of disturbing background are the reasons why HODS is used in these cases.

5.4 Nonspectroscopic Applications

The two major fields of nonspectroscopic applications of the derivative technique are chromatography and thermography (Table 5-54 and 5-57). In the former case, it is possible to improve the separation of unsatisfactory peaks and to identify the fractions. In the larger case, the fine structure of thermograms can be better evaluated. The first or second order derivatives are sufficient in most situations. This is also valid for electrograms and polarograms.

In conclusion, if there are but a few references concerning a special problem of interest, the reader should not be discouraged. On the contrary, this should provide a stimulus for developing new practical applications for higher-order differentiation.

5.5 Tables to Chapter 5

Table 5-1. UV–VIS spectrophotometry: inorganic cations, main group elements.

Substance (Cations)	Author	Year	d^n	Comments	Ref.
Be, Mg	Salinas	1987	1	Organic complex	1
Be	Zhu	1989	3	In beryl	2
Bi	Morelli	1982	1, 2	Complex with thiobarbituric acid; Cu present	3
Bi, Pb	Li	1986	4	Traces in Sn	4
Ca	Chen	1986	4	Organic complex	5
Ca	Jimenez	1990	–	In blood and urine	6
Ge	Wang	1987	–	Traces in minerals	7
Mg	Salinas	1986	1	Organic complex	8
Pb (Zn, Cd)	Li	1989	3	In surfactants	9
S	Niu	1985		Indirect determination in cluster compounds	

Table 5-2. UV–VIS spectrophotometry: transition elements not including lanthanides and actinides.

Substance (Cations)	Author	Year	d^n	Comments	Ref.
Au, Pd, Pt	Kuroda	1990	1	Chlorocomplexes	11
Cd, Zn	Talsky	1981	5	Mixtures	12
Cd, Zn, (Pb)	Li	1989	3	In surfactants	9
Co	Singh	1984	2	In p.p.b. concentrations	13
Co	Spitsin	1985	–	In alloys	14
Co, Cu	Bermejo-Barrera	1985		EDTA complex	15
Co	Odashima	1986		Organic complex; traces	16
Co	Jyothi	1987	>2	Complexed with dye	17
Co, Fe	Jiang	1988		In Ni	18
Co, Ni	Murillo	1988	1	Simultaneous estimation	19
Co, V	Jimenez	1989	2	In steels	20
Cr(III)	Shijo	1986	2	Organic complex	21
Cr, Mn	Zhen	1986	–	In Cr steels	22
Cr, Mn	Iyer	1986	–	Simultaneous estimation; in steel	23
Cu	Ishii	1980	2, 4	Organic complex	24
Cu, Fe(III)	Morelli	1983	1, 2	Organic complex	25
Cu, Co	Bermejo-Barrera	1985	–	EDTA complex	15
Cu, Zn	Li	1987	–	Organic complex	26
Cu, Zn	Wei	1989	4	Organic complex; in water; in rare earths	27
Fe(II), Fe(III)	Talsky	1982	4	Directly estimated	28
Fe(III), Co, Ni	Talsky	1982	4	Direct and simultaneous estimation	28

Table 5-2. Continued.

Substance (Cations)	Author	Year	d^n	Comments	Ref.
Fe	Singh	1983	2	μg concentrations	29
Fe(III), Cu	Morelli	1983	1, 2	Organic complex	25
Fe(III)	Bermejo-Barrera	1984	–	EDTA complex	30
Fe(II), Fe(III)	Haubensak	1985	4	Directly estimated	31
Fe(III), Co, Ni	Haubensak	1985	4	Direct simultaneous estimation	31
Fe(III)	Bermejo-Barrera	1986	–	EDTA complex	32
Fe	Odashima	1986	–	Organic complex; traces	33
Fe	Ishii	1986	2	Organic complex	34
Fe(III), Bi	Bermejo-Barrera	1987		EDTA complex	35
Fe, Nd, Pr	Ren	1987	3	In Nd-Fe alloy	36
Fe, Co	Jiang	1988		In Ni	18
Fe(III)	Mori	1989	3	Organic complex	37
Hg(II)	Griffiths	1979	2, 4		38
Hg	Medilina	1986	–	Organic complex	39
Hg	Sharma	1989	–	In pesticides	40
In	Sharma	1986	>2	Organic complex	41
Mn, Cr	Zhen	1986	–	In Cr-steels	22
Mn, Cr	Iyes	1986	–	Simultaneous estimation; in steel	23
Mn	Lin	1987	–	Traces	42
Mn	Kus	1989	4	In Ni	43
Mo	Qu	1985	1	Organic complex; in ores and minerals	44
Mo	Hernandez-Mendez	1987	3	Dye complex	45
Mo, Ti, V	Suzuki	1987	1	Peroxo complexes; in mixtures	46
Nb	Wang	1988		In minerals	47
Ni	Ishii	1982	2	In p.p.b. concentrations	48
Ni, Co, Fe(III)	Talsky	1982	4	Direct simultaneous estimation	28
Ni, Co, Fe(III)	Haubensak	1985	4	Direct simultaneous estimation	31
Ni	Malinowska	1986	–	Organic complex	49
Ni	Wang	1986	–	In Co minerals	50
Ni, Co	Murillo	1988	1	Simultaneous estimation	19
Os(VIII), Pd(II)	Morelli	1985	2	Mixtures	51
Pd(II), Ru(III)	Morelli	1983	2	In mixtures	52
Pd, Pt	Qu	1984	1	Simultaneous estimation in ores	53
Pd(II), Os(VIII)	Morelli	1985	2	Mixtures	51
Pd, Pt	Kus	1987	5	Dithizonates	54
Pd, Pt	Mai	1987	–	Simultaneous estimation	55
Pd, Pt, Au	Kuroda	1990	1	Chlorocomplexes	11
Rh(III)	Shijo	1988	2	Organic complex	56
Ru(III), Pd(II)	Morelli	1983	2	Mixtures	52
Ru(III)	Shijo	1987	2	Organic complex	57
Sc	Li	1986	–	Dye complex	58
Sc	Li	1988	3	In alloys	59
Ta	Kvaratskheli	1990	1	In presence of Nb	60
Ti, V, Mo	Suzuki	1987	1	Peroxo complexes; mixtures	46

Table 5-2. Continued.

Substance (Cations)	Author	Year	d^n	Comments	Ref.
V, Co	Jimenez	1989	2	In steels	20
Zn, Cd	Talsky	1981	5	Dithizonates; mixtures	12
Zn, Cd	Talsky	1982	5	Dithizonates; in mixtures	61
Zn, Cu	Li	1987	–	Organic complex	26
Zn, Cd, Pb	Li	1989	3	In surfactants	9
Zn, Cu	Wei	1989	4	Organic complex	27
Zr	Wang	1986	1	In Ag-Pd alloys	62
Metal ions	Edwards	1985	–	In humic substances	63

Table 5-3. UV–VIS spectrophotometry: lanthanides.

Substance (Cations)	Author	Year	d^n	Comments	Ref.
Rare earths	Shibata	1973	1		64
Rare earths	Zhang	1986	>2	Organic complex	65
Ce, Er	Poro	1972	1	Organic complex	66
Ce, Tb	Mishchenko	1987	–		67
Eu, Sm	Kucher	1983	–	In mixtures	68
Eu, La	Rao	1986	>2	Simultaneous estimation	69
Gd	Lepine	1986	2	In nitrate salts	70
Gd	Alexandrova	1988	–		71
Gd	Yan	1989	4	In rare earths	72
Ho	Cottrell	1980	2		73
La, Eu	Rao	1986	>2	Simultaneous estimation	69
Nd	Hernandez-Mendez	1987	>2	Organic complex	74
Nd, Tm	Garcia Sanchez	1987	–	Mixtures	75
Nd	Hernandez-Mendez	1988	>2	Complexed; in glasses	76
Ho, Nd, Sm, Er, Eu, Pr	Li	1989	3		77
	Alexandrova	1982	–	EDTA complex	78
Pr, Nd, Sm, Eu, Dy, Ho, Er, Tm	Ren	1985	>2	Mixtures of rare earths	79
Pr, Nd, Dy, Tm	Alexandrova	1987	–	In mixtures	80
Pr, Nd, Sm, Eu, Ho, Er	Bai	1987	–	EDTA complexes	81
Pr, Nd, Eu, Ho, Er, Tm	Chen	1987	2	Organic complexes	82
Pr	Sukumar	1988	3	Traces in Al	83
Sm	Bhagarathy	1988	3	Dye complex	84
Tm, Nd	Garcia Sanchez	1987	–	Mixtures	75

Table 5-4. UV-VIS spectrophotometry: actinides.

Substance (Cations)	Author	Year	d^n	Comments	Ref.
U(IV)	Perfil'ev	1984	–	Organic complex	85
$(UO_2)^{2+}$	Perfil'ev	1986	–	Organic complex	86
$(UO_2)^{2+}$	Skujins	1986	2		87
U	Kvaratskheli	1988	1	Th present	88
Th, U	Kuroda	1990	2	Arsenoazo-complex	89

Table 5-5. UV-VIS spectrophotometry: inorganic anions.

Substance (Anions)	Author	Year	d^n	Comments	Ref.
Borate	Wollin	1988	2	Dye complex	90
CNO^-	Gans	1983	4	Limiting concentration 10^{-2}M	91
CrO_4^{2-}	Cottrell	1980	2, 4	Spectra	73
$Cr_2O_7^{2-}$	Talsky	1982	1-6	Spectra	61
F^-	Ferris	1988	–	La complex	92
MnO_4^-	Talsky	1978	1-9	Spectra	93
MnO_4^-	Cottrell	1980	2	Spectra	73
NO_2, NO_3^-	Nagashima	1986	2	Limiting concentration 10^{-8}	94
	Suzuki	1987	2	Simultaneous determination	95
NO_3^-	Shimizu	1988	2	In water	96
	Wollin	1990	1-3	In water	97
P	Guo	1986	–	In copper	98
PO_4^{3-}	Harmsen	1984	–	Turbid solution	99
SO_4^{2-}	Ding	1988	1	In acid rain; in air	100

Table 5-6. UV-VIS spectrophotometry: minerals and other inorganic solids.

Substance	Author	Year	d^n	Comments	Ref.
$CoAl_2O_4$, Fe_2O_3	Cottrell	1980	2	Reflectance of powder samples	73
Fe oxides	Kosmas	1984	2	Mineral powders	101
Fe_2O_3	Haubensak	1985	2, 4	Dependence of absorbance from Specific area of the sample	31
Geothides, hematites	Kosmas	1986	2	Substitution of Al	102
Layer silicates	Talsky	1982	1-6	Transmission spectra of powders and tablets	28
	Talsky	1982	1-6	Different activated Bentonites; Co(II) adsorbed on Montmorillonite	103
	Haubensak	1985	1-6	Transmission spectra of powders and tablets; activated Bentonites; Co(II) adsorbed on Montmorillonite	31
$LiNbO_3$	Krivo Sodrov	1985	–	Er complex	104
	Born	1989	4	Estimation of Li_2O mole fraction	105

Table 5-6. Continued.

Substance	Author	Year	d^n	Comments	Ref.
Metal alloys	Stearns	1982	2	Optical properties	106
$NH_4Fe(SO_4)_2$	Haubensak	1985	2, 4	Transmission spectra of powders in KBr and polyethylene tablets	31
$(NH_4)_2Fe(SO_4)_2$ Solids (general)	Talsky	1988	1-6	Transmission and reflection spectra of optical filters, powders, suspensions, DC spots	107
	Talsky	1978	1-6	Different techniques for investigation of solids	108

Table 5-7. UV-VIS spectrophotometry: inorganic gases and vapors.

Substance	Author	Year	d^n	Comments	Ref.
CO, NH_3, NO, NO_2 O_3, SO_3	Williams	1970	2	Air pollutants	109
HCl	Pokrowsky	1984	–	Sensitive detection	110
I_2, SO_2	Schmitt	1977	2	Spectra	111
NH_3	Grum	1972	2	In automobile exhaust; air pollutant	112
NH_3	Hager	1970	2	In blood and respired air	113
	Izumi	1981	2	In smokestack emissions	114
	Bigelow	1983	2	In soils	115
NO, NO_2, SO_2	Hager	1973	2	Gas analysis	116
NO_2, SO_2, SO_3	Penzhorn	1983	2	Thermal and photochemical reaction of NO_2 with SO_2 and SO_3	117
O_3, SO_2	Talsky	1980	4, 6	In air and water	93
Multicomponent gases	Sagawa	1979	2	Simultaneous determination	118
	Sagawa	1985	2	Simultaneous determination of air polluters	119
Gases and vapors (general)	Talsky	1978	1-6	Different techniques	108

Table 5-8. UV-VIS spectrophotometry: alkaloids.

Substance group	Author	Year	d^n	Comments	Ref.
Alkaloids (see also pharmaceuticals)	Fell	1978	2, 4	Pilocarpine HCl, hyoscine HBr, eserine H_2SO_4 in drugs	120
	Cottrell	1980	2	Amphetamine, diphenhydroxamine	73
	Bettero	1985	–	Atropine	121
	Wang	1988	2	In plants	122
	Onur	1989	3	Ephedrine	123
	Onur	1989	4	Ephedrine	124

Table 5-9. UV-VIS spectrophotometry: amino acids.

Substance group	Author	Year	d^n	Comments	Ref.
Amino acids	Brandts	1973	2	Tyr in some proteins	125
	Mayring	1977	1-4	Spectra of Phe, Tyr, Trp; Phe in Tyr/Trp mixtures	126
	Talsky	1978	1-4	Mixtures	108
	Fell	1979	2, 4	Single amino acids; mixtures of Phe, Tyr, Trp	127
	Fell	1979	2, 4, 6	Single amino acids; mixtures of Phe, Tyr, Trp	128
	Ichikawa	1979	2	Phe in proteins	129
	Cottrell	1980	2, 4	Mixtures in sera	73
	Bell	1981	1	Phe in water and DMS	130
	Ichikawa	1981	2	Mixtures	131
	Fell	1981	2, 4, 6	Single amino acids; ternary mixtures	132
	Pye Unicam	1981	4	Phe, Tyr, Trp spectra	133
	Kullman	1982	–	Phe in serum	134
	Levine	1982	2	Aromatic residues in proteins	135
	Padros	1982	4	Phe in proteins	136
	Servillo	1982	2	Tyr, Trp in proteins	137
	Yamagishi	1982	2	Amino acids in soybean	138
	Dunach	1983	4	Trp in proteins	139
	Tichy	1983	1-5	Phe, Tyr, Trp in mixtures	140
	Levine	1984	1	Quantitative separation of PTH amino acids	141
	Talsky	1984	1-4	Amino acids in mixtures and in proteins	142
	Xu	1988	3	Phe, Tyr, Trp in injection preparations	143
	Zhu	1989	3	Trp in complex amino acid injections	144
	Saakov	1990	2	Aromatic amino acids	145

Table 5-10. UV-VIS spectrophotometry: antibiotics.

Substance group	Author	Year	d^n	Comments	Ref.
Antibiotics	Johnes	1981	2	Procyclidine in tablets and injections	146
	Riedl	1984	–	Natamycin in cheese	147
	Kovacs-Hadady	1988	4	Impurities in penicillins	148
	Wahbi	1988	1	Tetracyclines	149
	Murillo	1990	2	Amoxicillin, cephalexin	150

Table 5-11. UV-VIS spectrophotometry: chlorophylls and other plant pigments.

Substance group	Author	Year	d^n	Comments	Ref.
Chlorophylls and other plant pigments	French	1958	1	Chlorophylls, carotinoids	151
	Meister	1966	2	Plant pigments	152
	Cramer	1968	–	Chlorophylls at low temperatures	153
	Litvin	1969	4	Plant pigments	154
	Butler	1970	2, 4	Photosynthetic material	155
	Machold	1971	2	Chlorophyll electropherograms and TLC plates	156
	Saakov	1973	2	Chlorophyll a and b; blood pigments	157
	Litvin	1975		Chlorophyll a aggregates	158
	Whitten	1978	8	Bacteriochlorophyll	159
	Giller	1980	–	Synthetic chlorophyll-protein complexes	160
	Talsky	1980	4	Bacteriochlorophyll-protein complexes	161
	Navarro	1982	2	Chlorophylls	162
	Almela	1983		Pheophitin a	163
	Purohit	1983	2	Chlorophyll degradation; chlorophyllase	164
	Meck	1984	–	Zeaxanthin, lutein, chlorophyll a and b	165
	Daley	1986	4	Leaf lamina	166
	Daley	1987	4	Fragaria clones	167
	Talsky	1988	4, 8	Reflection spectra of living plants	168
	Talsky	1989	4	Reflection spectra of living plants	169
	Schmid	1990	4	Reflection spectra of leaves	170
	Wollin	1990	5	Chlorophyll and pheophytin in surface water	171

Table 5-12. UV-VIS spectrophotometry: cosmetics.

Substance group	Author	Year	d^n	Comments	Ref.
Cosmetics	Crane	1978		Lipsticks, face powders, pigments (make-up)	172
	Etournaud	1984		Nail polish	173

Table 5-13. UV-VIS spectrophotometry: cytochromes.

Substance group	Author	Year	d^n	Comments	Ref.
Cytochromes	Talsky	1978	3	Cytochrome c spectrum	108
	Ruckpaul	1980	2	Liver microsomal cytochrome	174

Table 5-14. UV-VIS spectrophotometry: beverages.

Substance group	Author	Year	d^n	Comments	Ref.
Beverages	Schmitt	1977	2	Quinine in drinks; caffein in Cola; wine, orange juice	111
	Talsky	1978	4	Types of beers; types of milk; saccharin in drinks and limonade; wine; teas; cacao; liquers; fruit juices	108 93
	Talsky	1981	4	Saccharin in drinks, lemonade	12
	Talsky	1982	4	Caffein in coffee extract	175
	Creus	1984	2	Malvin in wine	176
	Skujin	1986	2	Cocacola, Pepsi Cola; herbal tea, camomile tea, mint tea; Blue Curacao, liqueurs, Fernet Branca, Menta	87
	Humphrey	1987	2	Coffee extract, instant coffee, chicory	177
	Urena Pozo	1987	–	Ga, Zn in wine, drinking water	178
	Abdel-Moety	1988	1	Caffein in coffee, tea, soft drinks	179
	Deng	1988	2	Caffein in Cola drinks	180
	Iza	1988	2	Self-association of caffein in aqueous solution	181

Table 5-15. UV-VIS spectrophotometry: narcotics.

Substance group	Author	Year	d^n	Comments	Ref.
Narcotics (see also Pharmaceutics)	Lopez	1982	2	Morphine, heroin	182
	Gao	1983	2	Drug analysis	183
	Lawrence	1984	2	Heroin-morphine mixtures	184
	Arufe-Martinez	1988	2	Cocaine	185
		1989	2	Cocaine and other local anesthetics	186

Table 5-16. UV-VIS spectrophotometry: colorants.

Substance group	Author	Year	d^n	Comments	Ref.
Dyestuffs and other colorants	Botton	1975	2	Dyestuffs with background turbidity	187
	Botton	1977	2	Food colorants	188
	Sasaki	1978	2	Food colorants	189
	Talsky	1978	1-4	Congo Red, spectra	190
	Talsky	1978	1-4	Congo Red, influence of derivative parameters	108
	Fell	1981	4	In pharmaceuticals	191
	Talsky	1981	4	Congo Red-Cresol Red mixture	12
	Talsky	1981	1-4	Blue dyestuffs	12
	Talsky	1981	1-6	Methylene Blue	192
	Heidecke	1983	4, 5	Azorubin and Naphthol Red	193
	Talsky	1983	1-6	Methyl Red spectra	194
	Etournaud	1984		In cosmetics	173
	Kadin	1984	2	Phenol Red polyacrylamide	195
	Bridge	1985	–	Acid dyes in fibers	196
	Chung-Pui	1985	–	Dye in hydrocarbon oils	197
	Fompeydie	1985	–	Purity of eosin	198
	Lezerovich	1985	–	Colorants in soybean	199
	Sasaki	1985	1-13	11 food colors	200
	Arcoria	1986		On polyester films	201
	Humphrey	1987	4	Tartrazine	177
	Ramanathan	1987		Colored solutions	202

Table 5-17. UV-VIS spectrophotometry: enzymes.

Substance group	Author	Year	d^n	Comments	Ref.
Enzymes (see also proteins	Brandts	1973	2	Tyr in RNase and some proteins	125
	Matsushima	1975	–	RNase, lysozyme, α-chymotrypsin; band shift	203
	Demchenko	1978	2	Perturbation effects in proteins	204
	Talsky	1978	4	Solution, powder, KBr tablets of trypsin	108
	Talsky	1978	4	Trypsin, chymotrypsin	190
	Talsky	1978	5	Trypsin	205
	Talsky	1978	1-7	RNase	108
	Talsky	1978	3	Katalase, aldolase, cytochrome, trypsin (native and denaturated)	108
	Fell	1979	4, 6	Enzymes and biological materials	127
	Talsky	1979	4	Trypsin in solution, powdered, and in KBr tablets	206
	Talsky	1979	2, 4	LDH isoenzymes and LDH from different species	207
	Cottrell	1980	2, 4	RNase, trypsin	73

Table 5-17. Continued.

Substance group	Author	Year	d^n	Comments	Ref.
	Bell	1981	1	lysozyme in guanidine-HCl	130
	Fell	1981	4	DNase, RNase	132
	Talsky	1981	4	Trypsin, reaction in ice	208
	Naundorf	1982	4	Alkaline phosphatase	209
	Talsky	1982	3	Trypsin solution; KBr tablets ob trypsin powder and of CMC trypsin	210
	Talsky	1982	5	LDH isomeric forms	210
	Talsky	1982	3	Trypsin, ditrypsin; influence or organic solvents	210
	Morse	1983		Isoproteins	211
	Talsky	1983	4	Chymotrypsin oligomers	212
	Talsky	1983	1-6	Urease spectra	194
	Talsky	1984	2	Modified chymotrypsins	213
	Talsky	1984	4	Chymotrypsin oligomers	214
	Haubensak	1985	2, 4	Immobilized trypsin and chymotrypsin	31
	Talsky	1986	2, 4	Immobilized trypsin and chymotrypsin	215
	Kulig	1988	–	Monomer and dimer elastase	216

Table 5-18. UV-VIS spectrophotometry: foods.

Substance group	Author	Year	d^n	Comments	Ref.
Foods (see also beverages)	Schmitt	1977	2	Milk; polyenes in vegetable shortening	111
	Talsky	1978	4	Fats and oils	108
	Cottrell	1980	2	Milk	73
	Perkin-Elmer	1980	2	Olive oil, linseed oil	217
	Simal	1982	2	Benzoic acid in foods	218
	Riedl	1984		Natamycin in cheese	147
	Skujins	1986	2	Olive oil	87
	Kapoulos	1987	2	Olive oil adulteration	219
	Luf	1988	–	Norbixin and bixin in cheese	220
	Calapaj	1990	2	Vegetable oils	221

Table 5-19. UV-VIS spectrophotometry: hemoglobins.

Substance group	Author	Year	d^n	Comments	Ref.
Hemoglobin	Taulier	1981	1	Methemoglobin in blood	222
	Talsky	1982	4	Hemoglobin (human)	223
	Talsky	1983	4, 5	Hemoglobins (human and bovine)	194
	Weisser	1983	2	Methemoglobin in plasma	224
	Siek	1984	–	Carboxyhemoglobin	225
	Fukui	1985	–	Carboxyhemoglobin in blood	226
	Heales	1985	1	Methemoglobin	227
	Parks	1985	2	Carboxyhemoglobin	228
	Sanderink	1985	–	Hemoglobin in plasma	229
	Yang	1985	–	Carboxyhemoglobin in blood	230
	Soloni	1986	–	Hemoglobin in plasma	231

Table 5-20. UV-VIS spectrophotometry: homologs and isomers.

Substance group	Author	Year	d^n	Comments	Ref.
Homologs and Isomers	Porro	1972	–	Iso and terephthalic acid	66
	Dubrovkin	1978	1	Dichlorophenol isomers	232
	Talsky	1978	3, 4	Aliphatic alcohols, acetic acid esters, aromatics	108, 93
	Hewlett-Packard	1983	2	Chlorophenols, o- and p-cresol	233

Table 5-21. UV-VIS spectrophotometry: hormones.

Substance group	Author	Year	d^n	Comments	Ref.
Hormones (see also steroids)	Korany	1985	2	Ethinylestradiol, norethisterone	234
	Skujins	1986	–	Testosterone	87
	Widjaja	1986	–	Ethinylestradiol	235

Table 5-22. UV-VIS spectrophotometry: myoglobins.

Substance group	Author	Year	d^n	Comments	Ref.
Myoglobin	Talsky	1980	5	Differences in whale and horse myoglobin spectra	236
	Talsky	1982	5	Comparison of whale and horse myoglobin spectra	210
	Ragone	1987	2	Unfolding of myoglobin by denaturants	237

Table 5-23. UV-VIS spectrophotometry: nucleic acids and their bases.

Substance group	Author	Year	d^n	Comments	Ref.
Nucleic acids and their bases	Talsky	1980	3	RNA spectrum	236
	Bell	1981	1	Different nucleic acids	130
	Talsky	1982	3	Nucleic acids and adenosine-5-monophosphate	210
	Gil	1983	–	Adenine and cytosine spectra	238
	Sly	1986	2	Guanine and cytosine in DNA	239

Table 5-24. UV-VIS spectrophotometry: pesticides.

Substance group	Author	Year	d^n	Comments	Ref.
Pesticides	Porro	1972	1	Methylpyridinium chloride on clay	66
	Weigert	1981	1	4-Hydroxycoumarins	240
	Garcia-Sanchez	1988	–	Pesticide residue mixtures	241

Table 5-25. UV-VIS spectrophotometry: pharmaceuticals.

Substance group	Author	Year	d^n	Comments	Ref.
Pharmaceuticals (including alkaloids)	Elsayed	1977	1	Theobromine-sodium salicylate	242
	Schmitt	1977	2	Caffein, quinine	111
	Fell	1978	2	Phenol and aromatic alcohols in oily injections	243
	Talsky	1978	5	Mixtures of vitamin C and cysteamine	108
	Fell	1979	4, 6	Separation of phenol and p-cresol	128
	Fell	1980	2	Sulfoxide in degraded chloro-promazine formulations	244
	Fell	1981	2, 4	Amaranth, carmoisine, sunset yellow, tartrazine, green S in pharmaceuticals	191
	Fell	1981	2, 4	Paraquat	245
	Talsky	1981	4	Azulene in camomile oil	93
	Weigert	1981	1	4-Hydroxycoumarins	240
	Lawrence	1982	2	Amphetamine, phenylethylamine phentermine, ephedrine, meperi-dine (forensic analysis)	246
	Lopez	1982	2	Sorbic and benzoic acid	247
	Talsky	1982	4	Phytopharmacum	175
	Hewlett-Packard	1983	2	Flavonoids and guyazulene in camomile oil	233
	Kitamura	1983	2	Salicylic acid in aspirin	248

Table 5-25. Continued.

Substance group	Author	Year	d^n	Comments	Ref.
	Abdel-Hamid	1984	2	Benzodiazepines	249
	Davidson	1984	2	Benzenoid drugs in tablets and capsules	250
	Hassan	1984	2	Tropan	251
	Korany	1984	2	Eugenol, thymol	252
	El-Yazabi	1985	2	Nitracepam, chloracepate	253
	Fasanmade	1985	3	Chlorpromazine	254
	Lawrence	1985	2	Cannabinol, 9-terahydrocannabinol	255
	Abdel-Moety	1986	1	Caffein, procain (novocain) in geriatrics	256
	Arnoudse	1986		Theophylline (selective estimation)	257
	El-Sebakhy	1986	1	Quinine-quinidine	258
	Korany	1986	2	Acetaminophen, phenacetin	259
	Meal	1986	2	Arson analysis	260
	Sun	1986		Sodium benzoate – caffein injection	261
	Tang	1986	2	Bromhexin	262
	Atasoy	1987	2, 4	Paraquat	263
	Carlucci	1987	3	Indomethacin	264
	Fuke	1987	2	Paraquat and diquat in serum and urine	265
	Kitamura	1987	2	Isoniazid	266
	Knochen	1987	2	Homatropine	267
	Tao	1987	2	Bromhexine in cough tablets	268
	Aaron	1988	1, 2	Purines, pyrimidines	269
	Abdel-Hamid	1988	4	Diazepam, oxazepam	270
	Davidson	1988		Tripolidine, pseudoephedrine, dextromethorphan	271
	Dingeon	1988	2	Aceaminophen	272
	Iza	1988	2	Self-association of caffein in water	181
	Lin	1988	2	Lecithin	273
	Murtha	1988	2	Pseudoephedrine, chlorpheniramine, dextromethophan	274
	Vetuchi	1988		p-aminosalicylic acid, m-aminophenol	275

Table 5-26. UV-VIS spectrophotometry: synthetic organic pigments.

Substance group	Author	Year	d^n	Comments	Ref.
Pigments (synthetic organic)	Talsky	1987	1-6	DMSO solutions or thin layers of pigments for art paints	276
	Talsky	1989	4	Reflection spectra of art pigments (fiber cable optic)	277
	Ristić-Šolajić	1990	1-6	Investigations of synthetic organic pigments in DMSO solutions, thin layers, and reflection	278

Table 5-27. UV-VIS spectrophotometry: plant pigments.

Substance group	Author	Year	d^n	Comments	Ref.
Plant Pigments (see also Chlorophylls, Cytochromes)	Litvin	1969	2	Spectra	279
	Saakov	1978	2	Physiological investigations of pigments	280
	Saakov	1978	2	Pigment complex photosystem I	281
	Asadov	1985	–	Pigments in the thylakoid membrane	282
	Sidelnikov	1985	4, 8, 12	Rhodospirilum rubrum chromatophores	283
	Fisher	1987	4	Biotypes of Itchgrass	284

Table 5-28. UV-VIS spectrophotometry: polymers.

Substance group	Author	Year	d^n	Comments	Ref.
Polymers (synthetic)	Pump	1979	2	Pigments and chlorophenols in polyethylene	285
	Talsky	1979	4	Azo group determination in polycarbonates	286
	Cahill	1980	4	Polystyrene film	287
	Nuyken	1980	4, 5	Decomposition kinetics of azo groups; unconverted monomers; additives	288
	Talsky	1980	2, 4	Polyvinylpyrrolidone	236
	Allen	1981		Polymer films	289
	Soucek	1982	2	Phenolic antioxidants in polypropylene	290
	Sugai	1982	–	Conformational transition of copolymers	291
	Talsky	1982	4, 5	Residual monomers in polymers; polyvinylpyrrolidones with different molar masses	210
	Morse	1984	–	Epoxy resin analysis	292
	Sastre	1984	2	PVC degradation	293
	Skujin	1986	2	Plastic films	87
	Mori	1987	–	Polymer analysis	294
	Mouri	1988	4	Polystyrene	295

Table 5-29. UV-VIS spectrophotometry: porphyrins.

Substance group	Author	Year	d^n	Comments	Ref.
Porphyrin (without chlorophyll)	Johnes	1976	2	Porphyrins, porphyrinogens in urine	296
	Cook	1977	1	Bilirubin in albumin solutions	297
	Schmitt	1977	2	Porphyrins in urine	111
	Schmitt	1977	2	Urinoporphyrins	298
	Johnes	1979	2	Urinary porphyrins	299
	Talsky	1979	4	Porphyrins in urine	207
	Caselli	1981		Bilirubin	300
	Fell	1983	4	Ratio of copro- and uroporphyrins	301
	Talsky	1983	4	Porphyrins in urine	194
	Nolte	1985	–	Total porphyrins in urine	302
	Walters	1986	–	Multiwavelength analysis of differentiated spectra of urinary porphyrins	303

Table 5-30. UV-VIS spectrophotometry: proteins.

Substance group	Author	Year	d^n	Comments	Ref.
Proteins (see also enzymes)	Brandts	1973	1	Tyr in RNase, inhibitors, insulin; solvent effects	125
	Matsushima	1975	1	Insulin, RNase A, lysozyme, chymotrypsin	203
	Cook	1977	1	Bilirubin in albumin solutions	297
	Ichikawa	1977	2	Phe in proteins	304
	Schmitt	1977	2	Bovine albumin	111
	Balestrieri	1978	2	Aromatic aminoacids in proteins (catalase, RNase, lysozyme)	305
	Demchenko	1978	2	Aromatic aminoacids in proteins; solvent perturbation effect on lysozyme, aldolase, serum albumin	204
	Talsky	1978	4	Trypsin spectrum	190
	Talsky	1978	3	Spectra of RNase, γ-globulin, serum albumin, fibrinogen, aldolase, cytochrom c, hen albumin, methylated hen albumin	108
	Demchenko	1979	2	Proteins in turbid preparations	306
	Ichikawa	1979	2	Phe in proteins (lysozyme, insulin, RNase, serum albumin)	129
	Talsky	1979	4	Phe, Tyr, Trp in proteins, sera, enzymes	207
	Balestrieri	1980	2	Tyr in proteins	307
	Cahill	1980	4	Protein fingerprints	287
	Ruckpaul	1980	2	Conformational changes in hemoproteins	308
	Fell	1981	1-6	Aromatic aminoacids in RNase, DNase	132
	Ichikawa	1981	2	Effect of dodecylsulfate on Phe in serum albumin	309
	Jackson	1981	2	Protein concentrations	310
	Talsky	1981	1-6	Spectra of bovine serum albumin	192
	Irace	1982	2	Protein conformation changes	311
	Naundorf	1982	4	Bovine albumin	209
	Padros	1982	4	Tyr and Phe in proteins	136
	Servillo	1982	2	Tyr and Trp in proteins	137
	Fell	1983	4	Protein analysis	301
	Padros	1984	4	Spectra of proteins	312
	Ragone	1984	2	Tyr exposure in proteins	313
	Deng	1986	–	Human serum albumin	314

Table 5-31. UV-VIS spectrophotometry: sera.

Substance group	Author	Year	d^n	Comments	Ref.
Sera (see also proteins, amino acids, clinical chemistry)	Talsky	1979	1-4	Serum proteins	207
	Cottrell	1980	2, 4	Human serum	73
	Kullman	1982		Phe in serum	134
	Dingeon	1988	2	Acetaminophen in serum	315

Table 5-32. UV-VIS spectrophotometry: organic solids.

Substance group	Author	Year	d^n	Comments	Ref.
Solids (organic)	Porro	1972	1	Methylpyridinium chloride on clay	66
	Talsky	1978	1-4	Trypsin powder	108
	Talsky	1979	4	Trypsin powder	206
	Talsky	1981	4	Dyes adsorbed on alumina	12
	Talsky	1982	4	CMC trypsin, CMC chymotrypsin	210
	Talsky	1987	4	Synthetic organic pigments in thin layers	276
	Talsky	1988	1-6	Optical glass and silica filters, powders, TLC spots, films, crystals	107
	Talsky	1989	4	Reflection spectra of pigments in thin layers	277
	Talsky	1989	4	Transmission and reflection spectra of leaves of living plants	169
	Ristić-Šolajić	1990	1-6	Transmission and reflection spectra of organic pigments in thin layers of art paints	278
	Schmid	1990	4	Transmission and reflection spectra of chlorophyll in leaves of various plants	170

Table 5-33. UV-VIS spectrophotometry: steroids.

Substance group	Author	Year	d^n	Comments	Ref.
Steroids (see also hormones)	Olson	1960	1, 2	Testosterone	316
	Fell	1982	4	In pharmaceutical formulations	317
	Vergeichik	1983	–	Sinestrol, octestrol	318
	El-Yazbi	1986	–	Corticosteroids	319

Table 5-34. UV-VIS spectrophotometry: vitamins.

Substance group	Author	Year	d^n	Comments	Ref.
Vitamins	Schmitt	1977	2	Vitamin A, B_1, B_2, B_6, B_{12}, C, D_2, E, K_3, P, rutin, folic acid, nicotinic acid	320
	Talsky	1978	5	Mixture of vitamin C and cysteamin	108
	Lage	1980	2	Vitamin C	321
	Such	1980	2	Vitamin mixtures	322
	Perkin-Elmer	1982	2	Vitamin A and E	323
	Fell	1983	4	Stability of vitamins	301
	Haines-Nutt	1984		Stability of vitamin K	324
	Deng	1986	2	Vitamin A and E	325
	Park	1986	–	Pyridoxin, nicotinamide	326
	Bukovits	1987	–	Tocopherols	327
	Park	1988	–	Vitamin B group and vitamin C	328
	Shi	1988	–	Folic acid	329

Table 5-35. UV-VIS spectrophotometry: various compounds.

Substance group	Author	Year	d^n	Comments	Ref.
Various organic compounds	Porro	1972	1	Iso-and terephthalic acid	66
	Shibata	1973	1	2,4-Dichloro and 2,4,6-trichlorophenol	64
	Botten	1977	2	Turbid suspension of aluminum silicate in benzyl alcohol	188
	Milano	1977	1	Benzanthracene, chrysene	330
	Schmitt	1977	2	Benzene in hexane; phenol in turbid water	111
	Dubrovkin	1978	1	Dichlorophenol isomers	331
	Fell	1978	2	Oily phenol injection	243
	Talsky	1978	2, 4	Benzene in ethanol	108
	Talsky	1978	3	Benzene in normal and super gasoline	108
	Fell	1979	2, 4	Mixtures of phenol and p-cresol	128
	Hawthorne	1979	2	Polynuclear compounds	332
	Cahill	1980	4	Phenol in broadband interference	287
	Fix	1980	1	Saccharin in watts nickelplating solution	333
	Lawrence	1980		naphthalene syntans	334
	Cottrell	1982	2	Anthracene	73
	Juffernbruch	1982	2	Quinoline, isoquinoline	335
	Vasileva	1982	–	3,4-Benzpyrene	336
	El-Din	1983	2	Cinamic aldehyde and carvone in volatile oils	337
	Ito	1983	2	Formaldehyde in exhaust gases	338

Table 5-35. Continued.

Substance group	Author	Year	d^n	Comments	Ref.
	Meal	1983	2	Ketones	339
	Ramanathan	1984	–	Benzyl alcohol in water and methanol	340
	Werle-Wilczynska	1984	–	Naphthalene, anthracene, pyrene in coal tars	341
	Yan	1984		Naphthol, naphthylesters	342
	Yan	1984		Anthracene in turbid solutions	343
	Dixit	1985	2	Spectra of hydrocarbons	344
	Montano Asquerino	1985		Polyphenols in olive pulp	345
	Tahboub	1985	2	Polynuclear aromatic hydrocarbons	346
	Dixit	1986	2	Alkylnaphthalenes in petroleum factions	347
	Di Pietra	1987	–	Benzaldehyd in benzyl alcohol	348
	Ivanovic	1990	3, 4	Antioxidants	349

Table 5-36. Other spectroscopic methods: Astronomical atomic absorption, auger electron, and ESR spectroscopy.

Application	Author	Year	d^n	Comments	Ref.
Astronomical spectroscopy	Bonoli	1983	–	–	350
Atomic absorption spectroscopy (AAS)	Walters	1966	1, 2	Fe and Al spectra	351
	Elser	1972	1, 2	Vidicon detector	352
	Ishii	1983	–	Rare earths in La oxide	353
	Zhang	1985	–	Mg estimation	354
Auger electron spectroscopy	Gilbert	1988	–	–	355
Electron spin resonance (ESR) spectroscopy	Bär	1975	1	Organic radicals	356

Table 5-37. Other spectroscopic methods: Flame emission, flow injection, and inelastic electron tunneling spectroscopy.

Application	Author	Year	d^n	Comments	Ref.
Flame emission spectrometry	Snelleman	1970	2	Ba in the presence of Ca	357
	Cook	1976	1	Na in water	358
Flow injection derivative spectroscopy	Ishii	1986	–	Trace amounts of Fe	34
Inelastic electron tunneling spectroscopy	Spanalescu	1981	2	–	359

Table 5-38. Other spectroscopic methods: Infrared and low-temperature spectroscopy.

Application	Author	Year	d^n	Comments	Ref.
Infrared spectroscopy	Collier	1956	2	o- and m-cresol, isomer phenols	360
	Collier	1959	2	CH_3 groups in polyethylen	361
	McWilliam	1967	1	Fine resolution of IR spectra	362
	McWilliam	1969	1	Acetone in chloroform; adipinonitrile in bezonitrile	363
	Keighley	1971	1		364
	Pump	1979	2	Double bonds in polymers	285
	Jungst	1981	2	Trace gas measurement	365
	Tallant	1981	2	Trace gas measurement	366
	Whitback	1981	1, 2	CO spectrum	367
	Sun	1983	2	Monomer-dimer equilibria of Perfluorocarboxylic acid	368
Low-temperature spectroscopy	Cramer	1968	–	Chlorophyll pigments in chloroplasts	369
	Talsky	1981	4	Trypsin catalysis in ice	208

Table 5-39. Other spectroscopic methods: Luminescence spectroscopy.

Application	Author	Year	d^n	Comments	Ref.
Luminescence spectroscopy (fluorescence and thermoluminescence)	Brown	1959	1	Several substances	370
	O'Haver	1972	1	Overlapping spectra	371
	Green	1974	2	Luminescence of benzypyrene, pyrene, anthracene oil	372
	O'Haver	1976	–	Technique	373
	Guliev	1978	–	Chloroplast fragments	374
	Miller	1979	2	Technique	375
	Vo-Dinh	1979	–	Technique	376
	Miller	1980	2	Proteins	377
	White	1980	–	Luteinizing hormone	378
	Fell	1981	2	Phe, Tyr, Trp	132
	Christenson	1982	–	Ratios metanephrine/normetanephrine and epinephrine/norepinephrine	379
	Garcia-Borron	1982	2	Trp and Tyr in proteins	380
	Miller	1982	2	Fluerescence in water and serum; Tyr and Trp; binding of fluorescence probes to macromolecules; mixtures Phe, Tyr, Trp	381

Table 5-39. Continued.

Application	Author	Year	d^n	Comments	Ref.
	Yamagishi	1982	2	Aromatic amino acids in soybean 7 S globulin	138
	Moreno-Moreno	1983	–	Thermoluminescence	382
	Allen	1984	–	Polyolefins	383
	Birmingham	1984	2	Membrane protein conformational changes	384
	Rubio	1985	–	Mixtures of Ti, Zr, Hf	385
	Gerow	1986	–	Background subtraction in TLC	386
	Gutierrez	1987	–	Histamine	387
	Urena	1987	–	Ga and Zn in biological samples, wine, drinking water, waste water	388
	Munoz de la Pena	1988	1	Salicylic and salicyluric acid	389
	Cano Pavon	1990		Ga and Al in blood, brain kidney and liver	390

Table 5-40. Other spectroscopic methods: Mass, mössbauer, and multichannel spectroscopy.

Application	Author	Year	d^n	Comments	Ref.
Mass spectroscopy	Beynon	1958	–	–	391
Mössbauer spectroscopy	Bressani	1967	–	–	392
Multichannel	Fell	1980	2, 4	Anthracene, phenanthrene	393
spectroscopy	Fell	1982	2, 4	6-acetylmorphine, procaine, caffein	394

Table 5-41. Other spectroscopic methods: Multiwavelength analysis, NMR, raman spectroscopy, and surface photovoltage spectroscopy.

Application	Author	Year	d^n	Comments	Ref.
Multiwavelength analysis of derivative spectra	Willis	1981	1, 2, >2		395
	Walters	1986	2	Porphyrins in urine	303
	Dingeon	1988	2	Acetaminophen in serum	315
Nuclear magnetic resonance (NMR)	Townes	1955	1, 2	–	396
	Bonfiglioli	1963	1	–	397
Raman spectroscopy	Heritage	1980	–	Carbonate on silver	398
	Gans	1982	–	Generation of spectra	399
	Van de Ven	1984	–	Biomembranes	400
Surface photovoltage spectroscopy	Lagowski	1979	1	Study of absorption in semiconductors (Ga arsenite)	401

Table 5-42. Other spectroscopic methods: Reflectance spectroscopy.

Application	Author	Year	d^n	Comments	Ref.
Reflectance spectroscopy	Montegu	1979	1	Mg-Zn-Te semiconductors	402
	Cottrell	1980	2	Fe_2O_3, $CoAl_2O_4$; diffuse reflection	73
	Bridge	1985	–	Some acid dyes on wool and nylon	196
	Fisher	1987	4	Biotypes of itchgrass	284
	Talsky	1988	1-8	Bentonites, $LiNbO_3$, pigment layers, pigments in living plants	107
	Talsky	1988	1-8	Pigments in living plants	168
	Talsky	1989	4	Art paints	277
	Talsky	1989	4	Chlorophylls in living plants	169
	Talsky	1989	1-8	Synthetic organic pigments; plant pigments	403
	Ristić-Šolajić	1990	1-6	Synthetic organic pigments, art paints	278
	Schmid	1990	4	Plant pigments; influence of SO_2 on plants	170

Table 5-43. Other spectroscopic methods: Tunable infrared diode laser spectroscopy.

Application	Author	Year	d^n	Comments	Ref.
Tunable infrared diode laser spectroscopy	Reid	1978	2	Method	404
	Grieble	1980	2	Line profile determination	405
	Olson	1980	2	Line profile determination	406
	Jungst	1981	2	Trace gas measurement	365
	Pekrovsky	1981	–	Hydrogen chloride	110
	Mucha	1982	2	Trace gas analysis	407
	Mucha	1983	2	Moisture	408
	Sanoyl	1983	–	Description of the method	409
	Weitkamp	1984	2	Calibration	410

Table 5-44. Special fields of application: Turbid samples.

Application	Author	Year	d^n	Comments	Ref.
Turbid samples (background turbidity)	Botten	1975	2	Dyes	411
	Botten	1977	2	Milk; Al silicate suspended in benzyl alcohol	188
	Schmitt	1977	2	Phenol in turbid water	111
	Demchenko	1978	2	Turbid solutions	204
	Talsky	1978	3, 4	Phenol and aniline in wastewater	108
	Demchenko	1979	2	Turbid protein preparations	306
	Cahill	1980	4	Styrene in turbid water	287
	Cottrell	1980	2	Phenol in river water	73
	Talsky	1983	4	Phenol in silica suspension	412
	Yan	1984	–	Turbid anthracene solutions	343

Table 5-45. Special fields of application: biochemistry.

Application	Author	Year	d^n	Comments	Ref.
Biochemistry (see also amino acids, proteins	Ruckpaul	1980	2	Cytochrome P-450 in liver microsomal monooxygenase systems	174
	Miller	1982	2	Fluorescein in serum; fluorescence spectra of amino acids; binding of fluorescence probes to macromolecules	381

Table 5-46. Special fields of application: biological materials.

Application	Author	Year	d^n	Comments	Ref.
Biological materials (see also biochemistry)	Butler	1970	4	Chloroplasts	413
	Demchenko	1978	2	Biological membranes	204
	Butler	1979	4	Bovine heart mitochondria	414
	Lin	1982	–	Lignin and lignin model compounds	415
	Service	1982	2	Lignocaine in levulose infusion	416
	Terada	1983	–	Uncoupler of oxidative phophorylation	417
	Warth	1983	–	Dipicolinic acid in bacterial	418
	Brunning	1984	1, >2	Algae in lake sediments	419
	Razzhivin	1984	–	Structure of Rhodospirillum rubrum chromatophore	420
	Van de Ven	1984	–	Biomembranes	400
	Edwards	1985	–	Interaction between metal ions and humic substances	63
	Montano	1985	–	Polyphenols in olive pulps and fermentation brines	345
	Sidelnikov	1985	4, 8, 12	Investigation of Rhodospirillum rubrum	283
	Borisov	1987	–	Biological specimens	421
	Urena	1987	–	Ga and Zn in biological samples	388
	Jimenez	1990	–	Ga in blood and urine	6

Table 5-47. Special fields of application: clinical chemistry.

Application	Author	Year	d^n	Comments	Ref.
Clinical chemistry (see also sera, hemoglobin, myoglobin, porphyrins)	Hager	1970	2	NH_3 in blood and respired air	113
	Williams	1970	2	NH_3 in blood and respired air	109
	Schmitt	1977	2	Porphyrins in urine	298
	Talsky	1978	–	Cysteamine and vitamin C	108
	Johnes	1979	2	Urinary porphyrins	299
	Maurer	1979	1	Creatinine in urine	422
	O'Haver	1979	2	Clinical applications	423
	Talsky	1979	1-4	Sera, serum proteins	207
	Talsky	1979	1-4	Sera, serum proteins	93
	Talsky	1979	1-4	Porphyrins in urine	207
	Caselli	1981	–	Bilirubin in amniotic fluid	300
	Jarvie	1981	2	Paraquat in plasma	424
	Weigert	1981	1	Coumarins in organs and blood	240
	Bertrand	1982	2	Methemalbumin in serum	425
	Fell	1983	–	Porphyrins in urine	301
	Kullman	1982	–	Phe in serum (phenylketonuria)	134
	Perkin-Elmer	1982	2	Total porphyrin in urine	323
	Nagashima	1983	2	Urea estimation by urease	426
	Talsky	1983	4	Porphyrins in urine	194
	Weisser	1983	1, 2	Methemoglobin in plasma	224
	Kandrnal	1984	–	Theophylline in serum	427
	Nolte	1985	–	Total porphyrins in urine	302
	Poulou	1986	–	Nitrofurantoin in urine	428
	Restal	1986	–	Conformational transition in Ca-Mg adenosine triphosphatase	429
	Ghersi-Egea	1987	2	Cerebral cytochrome P-450	430
	Stroes	1987		Blood pigments	431
	Taulier	1987	1	Methemoglobin in blood	222
	Fernandez	1988	–	Plasma amitriptyline and perphenazine	432
	Green	1988	2	Percutaneous absorption of naphazoline and oxprenolol	433
	Hu	1988	1	Theophylline in plasma	434
	Munoz de la Pena	1988	1	Salicylic and salicyluric acid in urine	389
	You	1988	–	Bilirubin in artificial calculus bovis	435

Table 5-48. Special fields of application: conformational analysis.

Application	Author	Year	d^n	Comments	Ref.
Conformational analysis	Ruckpaul	1980	2	Hemoproteins	308
	Irace	1982	2	Proteins	311
	Sugai	1982	–	Copolymers of maleic acid and styrene	291
	Birmingham	1984	2	Membrane proteins	384
	Ragone	1984	2	Tyr exposure in proteins	313
	Restal	1986	–	Conformational transition in Ca-Mg adenosine triphosphatase	429
	Sabes	1987	4	Charge perturbation of Trp residues	436
	Kulig	1988	–	monomer and dimer elastase	216
	Iza	1988	2	Self-association of caffein in water	181
	Berendzen	1990	–	Myoglobins, IR temperature derivative spectra	437
	Garriga	1990	4	DNA conformation	438

Table 5-49. Special fields of application: environmental analysis.

Application	Author	Year	d^n	Comments	Ref.
Environmental analysis (see also gases, inorganics)	Williams	1970	2	CO, NH_3, NO, NO_2, O_3, SO_2, benzene in air; NH_3 in automobile exhaust; air samples from waste treatment, sludge storage tank and power plant boiler smokestack	109
	Hager	1973	2	NO, NO_2, SO_2, benzene (spectra and minimum detectability)	116
	Shibata	1973	1	Di- and trichlorophenols	64
	Shibata	1976	1	Di- and trichlorophenols in industrial waste	439
	Milano	1977	1	Benzanthracene and chrysene in atmospheric particulates	330
	Falanga	1978	2	Air pollution	440
	Reid	1978	2	Polynuclear aromatic hydrocarbons and other pollutants	441
	Talsky	1978	3, 4	Aniline and phenol in waste water	108

Table 5-49. (continued)

Application	Author	Year	d^n	Comments	Ref.
Environmental analysis (see also gases, inorganics)	Hawthorne	1984	2	Polynuclear aromatics in gases and solutions	332
	Sagawa	1979	2	Multicomponent gases	118
	Ishii	1980	4	Ultramicroanalysis of Cu in water	24
	Ishii	1980	–	Ultramicroanalysis of water	442
	Maurer	1980	1	Uric acid and creatinine in water and wastewater	443
	Izumi	1981	2	NH_3 in smokesstack emissions	114
	Maurer	1981	1	Uric acid in urban wastewater; NO_3^-/NO_2^- in natural water	444
	Perkin-Elmer	1982	1, 2	Phenol in water	445
	Talsky	1982	2, 4	Aniline, phenol, chlorophenols in industrial wastewater; polynuclear aromatics in air	446
	Talsky	1982	2, 4, 6	Cu^{2+}, Co^{2+}, Ni^{2+}, adsorbed on clay mineral surfaces	447
	Ito	1983	2	Formaldehyde in exhaust gases	338
	Penzhorn	1983	2	Thermal and photochemical reaction of NO_2 with SO_2 and SO_3	117
	Talsky	1983	4, 5	Aniline and phenol in industrial wastewater; pentachlorophenol water; aniline in turbid wastewater; spectra of some organic substances	412
	Brunning	1984	1, >2	Algae and phosphates in fresh water	419
	Hawthorne	1984	2	Phenols in wastewater	448
	Wu	1986	4	Phenol and aniline in wastewater	449
	Wollin	1988	2	Borate in fresh water	90
	Wollin	1989	2, 4, 5	Aniline, phenol in water and wastewater	450
	Wollin	1989	2, 4, 5	Aniline and phenol in water and wastewater	451
	Schmid	1990	4	Influence of SO_2 on plant pigments	170
	Wollin	1990	1-3	NO_3^- in fresh water	97
	Wollin	1990	2-5	NO_3^-, borate, phenol, aniline; tubid phenol	452

Table 5-50. Special fields of application: fermentation, and forensic analysis.

Application	Author	Year	d^n	Comments	Ref.
Fermentation	Woodrow	1984	–	Estimation of substrate and product concentrations	453
Forensic analysis (see also pharmaceutical, narcotics, clinical chemistry	Weigert	1981	1	Coumarine derivatives in organs and blood	240
	Gill	1982	2	Amphetamine in liver extract	454
	Lawrence	1982	2	Amphetamine, phenylethylamine, phentermine, meperidine	246
	Siek	1984	–	Carbonylhemoglobin; blood hemoglobin pigments	225

Table 5-51. Special fields of application: kinetics.

Application	Author	Year	d^n	Comments	Ref.
Kinetics	Watari	1968	–	Monitoring of kinetic reactions	455
	Lester	1970	–	Monitoring of kinetic reactions	456
	Talsky	1979	4	Thermolysis of monomer and polymer azo compounds	286
	Nuyken	1980	4, 5	Decomposition of azo groups in polymers	288
	Gauglitz	1981	1	Photoreaction of stilbene and photoisomeration of diphenylbutadiene	457

Table 5-52. Special fields of application: materials science.

Application	Author	Year	d^n	Comments	Ref.
Materials science (see also inorganics, polymers, organic solids)	Cardona	1969	1	Investigations of solid materials (solid state physics)	458

Table 5-53. Special fields of application: microanalysis.

Application	Author	Year	d^n	Comments	Ref.
Microanalysis (trace analysis) (see also inorganic cations and anions, environmental analysis)	Hawthorne	1978	2	Trace organic analysis	459
	Talsky	1978	2, 3, 4	Metal ions; benzene in ethanol; aniline and phenol in wastewater; arenes in waste gases; benzene in gasoline	108
	Ishii	1980		Ultramicroanalysis of water	442
	Jungst	1981	2	Trace gas analysis	365
	Talsky	1981	4, 5	Zn^{2+}; saccharin; adsorbed dyes	12
	Ishii	1983	–	microamounts of Ni	460
	Li	1986	4	Pb and Bi in tin	4
	Lin	1987		traces of Mn	42
	Talsky	1987	1–6	Synthetic organic pigments in DMSO solutions; developed TLC spots	276
	Wang	1988	–	Microamounts of Nb in rock	47

Table 5-54. Nonspectroscopic applications: chromatography.

Application	Author	Year	d^n	Comments	Ref.
Chromatography	Kambara	1961	1	GC	461
	Machold	1971	2	TLC plates	462
	Riggs	1971	–	GC	463
	Fox	1976	–	LC	464
	Milano	1976	1	HPLC	465
	McDowell	1977	1	HPLC	466
	Houpt	1977	2	GC	467
	Milano	1977	–	LC	330
	Talsky	1978	2, 4	LC	108
	Talsky	1978	2, 4	HPLC	93
	Talsky	1978	2, 4	LC	190 93
	Talsky	1978	2, 4	LC	205 93
	Fell	1979	1-4	HPLC	132
	Fell	1979	2, 4	HPLC	128
	Okamura	1979		GLC, HPLC, TLC	468
	Cottrell	1980	2	LC	73
	Zelt	1980		GLC, HPLC	469
	Fell	1982	2, 4	HPLC	394
	Talsky	1983	2	LC, peak purity	212
	Lagesson	1984	–	GC	470
	Grant	1985	–	Peak purity	471
	Gerow	1986	–	TLC	472
	Hearn	1988	–	LC	473
	Quaglia	1988	4	TLC	474
	Talsky	1989	4	TLC; reflection mode	277
	Ristić-Šolajić	1990	4	TLC; reflection mode	278

Table 5-55. Nonspectroscopic applications: densitometry, electroanalysis, and electrophoresis.

Application	Author	Year	d^n	Comments	Ref.
Densitometry	Cottrell	1980	2, 4	Densitometric scan of sulfur 35 autoradiograph	73
	Traveset	1981	–	–	475
Electroanalysis	Sidwell	1986	–	Estimation of inflection points	476
Electrophoresis	Machold	1971	2	Evaluation of electropherograms, identification of components	462
	Okamura	1979	1, 2	Investigations of electropherograms	468

Table 5-56. Nonspectroscopic applications: polarography and potentiometry.

Application	Author	Year	d^n	Comments	Ref.
Polarography	Davis	1953	–	Polarographic data evaluation	477
	Perone	1965	–	Evaluation of polarograms	478
	Evnes	1967	–	Evaluation of polarograms	479
Potentiometry	Durst	1967	1	Estimation of silver in 1 N H_2SO_4	480
	Amer	1988	1	Quinone-containing drugs	481

Table 5-57. Nonspectroscopic applications: thermal analysis.

Application	Author	Year	d^n	Comments	Ref.
Thermal analysis	Erdey	1956		Evaluation of the thermogram curve	482
	Campbell	1959	1	Estimation of inflection points	483
	Freeman	1959	1	Apparatus	484
	Garn	1965	1	Apparatus	485
	Schultze	1969	1	Apparatus	486
	Lóránt	1972		DTG, DTA, and TG of glutamic acid, γ-globulin, egg albumin	487
	Cuellar	1978		Evaluation of thermograms	488
	Pivec	1982	1	Melting curve of DNA	489

5.6 References to Chapter 5

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Appendix

Publications by Gerhard Talsky * on Derivative Spectrophotometry and Related Differentiation Techniques.

With Lothar Mayring. „Über die analoge on-line-Differentiation höherer Ordnung zur Feinauflösung von UV-Visible-Spektren und anderen elektrischen Meßsignalen". *Fresenius Z. Anal. Chem.*, 292, (1978): 233.

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Index

Derivative Spectrophotometry. By Gerhard Talsky

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